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About the cover:
Colocalization of P2X4 using Anti-P2X4 antibody (AAPR-002) (red) and P2X3 (green) subunits in the distal population of GPN neurons in situ by confocal immunofluorescence.
Special Acknowledgement to Professor Colin A. Nurse from the Department of Biology
McMaster University, Ontario Canada for allowing Alomone Labs to use part of a published figure as this Modulator’s cover page.
Tripping Over TRPC

Melanie Grably, Ph.D.

The canonical transient receptor potential channels (TRPCs) are non-selective cation channels that are expressed in a variety of multicellular organisms with different functions. The TRPC family can be divided by homology and function into four subfamilies: TRPC1, TRPC2 (a pseudogene in human), TRPC4/5 and TRPC3/6/7. They can form either homo- or heteromeric channels with individual properties. This brief report aims at summarizing advances made regarding this subfamily of TRP channels and mainly how Alomone Labs’ products have influenced the progress made.

TRPC1

TRPC1, the first channel to be uncovered in this family may be involved in Duchenne muscular dystrophy (DMD). Using the animal model of the disease, Gervásio et al. demonstrated that muscle tissues have increased protein levels of TRPC1 using Alomone Labs’ Anti-TRPC1 antibody (ACC-010) (Figure 1), which may reflect higher levels of intracellular Ca²⁺ when compared to their wild type counterpart. To provide further evidence that TRPC1 participates in the pathophysiology of Duchenne, the authors show that TRPC1 colocalizes and binds to caveolin-3 on the plasma membrane of muscle cells using Anti-TRPC1 antibody (Figure 2) and go on to show that Src kinase may be responsible for triggering TRPC1 dependent Ca²⁺ uptake.

TRPCs have for long been assigned to being essential components of store-operated calcium entry (SOCE) since this mode of intracellular calcium increase is positively mediated by IP3 and since TRPCs were also found to bind IP3²⁻³. This speculation has been and still is quite controversial. Jardin et al. take this notion one step further and show in Figure 3 that in human platelet cells, Orai1 and TRPC1 can be co-immunoprecipitated upon extensive depletion of intracellular Ca²⁺ stores which activates SOCE (using Anti-TRPC1 antibody for immunoprecipitating and/or immunodetecting TRPC1)⁴.

TRPC3

TRPC3, an additional member of the TRP channels family is expressed in both excitable and non-excitible cells. Roedding et al. were interested in studying its expression pattern in the brain as a function of age⁴. They subsequently used postmortem prefrontal cortex and cerebellum from subjects ranging from ages of 8 days to 83 years and monitored the expression of TRPC3 levels indicate that TRPC3 expression in the prefrontal cortex are higher in the neonates/infants age group than in the adolescent to adult age group (Figure 5a) suggesting that TRPC3 may be developmentally regulated in that area of the brain. On the other hand, TRPC3 expression in the...
cerebellum was similar throughout the age groups (Figure 5B) suggesting that TRPC3 may have a physiological role in the cerebellum throughout the lifespan.

As TRPC3 shares 75% homology with TRPC6 and TRPC3 could compensate for the loss of TRPC6 in trpc6−/− mice and since TRPC6 promotes proliferation of prostate cancer epithelial cells, Yang et al. investigated whether TRPC3 plays a role in cellular proliferation in human ovarian cancer. Indeed, using Alomone Labs’ Anti-TRPC3 antibody, they found that TRPC3 is highly expressed in these tumors (Figure 6). Knocking-down the expression of TRPC3 using siRNAs targeted against specific regions of TRPC3 in ovarian cancer cell lines (protein levels were subsequently verified by western blot using Anti-TRPC3 antibody), cell growth and tumor development were suppressed in cell lines and in nude mice respectively. These data strongly suggest that TRPC3 may play a role in the development and progression of human epithelial ovarian cancer.

In human coronary artery endothelial cells (HCAECs), ATP is released into the extracellular milieu (and known to exert a strong proinflammatory effect) in response to ischemia, hypoxia, mechanical or chemical stress and induces the expression of VCAM-1 (vascular cell adhesion molecule 1) which leads to increased cellular Ca2+ levels. It has been shown that HCAECs express all members of TRPCs, and that TRPC3 contributes to ATP-stimulated Ca2+ influx. These studies are reinforced by Alvarez et al. which measured a significant decrease in the elicited current induced by ATP in rat ventricular cardiomyocytes under whole cell patch clamp when Anti-TRPC3 antibody is added to the pipette solution (Figure 7). Recently, Smedlund et al. showed that TRPC3 contributes to the actions of ATP on VCAM-1 using part Anti-TRPC3 antibody to monitor TRPC3 expression in HCAECs as well as to verify TRPC3 protein levels followed by siRNA treatment. These findings underscore a potential novel function of TRPC3 within the context of development and progression of atherosclerotic lesions in coronary artery diseases. The authors also use Anti-TRPC1 antibody (#ACC-010), Anti-TRPC4 antibody (#ACC-018), Anti-TRPC5 antibody (#ACC-020) and Anti-TRPC6 antibody (#ACC-017) to show that TRPC channels are expressed to certain degrees on the surface of HCAECs.

**TRPC4**

Recently, a role in maintaining high intracellular Ca2+ levels in the myometrium (the smooth muscle of the uterine wall) during labor contractions was attributed to TRPC4. shRNA was used in order to reduce the expression of TRPC4 (while expression of TRPC4 was verified by western blot using the Anti-TRPC4 antibody), and following various treatments known to increase intracellular Ca2+...
concentrations through extracellular Ca²⁺ entry such as oxytocin (GPCR-stimulated), thapsigargin (store depletion-stimulated) and OAG (DAG-stimulated) found that Ca⁺⁺ entry through TRPC4 is stimulated by GPCR¹⁰.

**TRPC5**

Calcium plays an important role in axon outgrowth and growth cone motility. Currents involving Ca²⁺ are significantly linked to axon formation. In addition, TRPC channels have been found implicated in Ca⁺⁺-dependent growth cones dynamics¹¹. In a recent paper, using rat embryonic hippocampal neurons, Davare et al. show that TRPC5 channels activate CaMKII/CaMKIγ in a Ca⁺⁺-dependent manner to induce axon formation¹². They show this elegantly and do so by using different approaches. One such approach was using siRNA to specifically knockdown TRPC5 and observing the effect on axon formation. In the first stage of the experiment, the authors verified that protein levels of TRPC5 are decreased upon siRNA treatment by staining electroporated cells with Anti-TRPC5 antibody and comparing the intensity between siTRPC5 treated and control siRNA treated cells (Figure 8A). The decrease in protein levels was indeed sufficient to significantly affect axon formation but downregulation of TRPC6 by siTRPC6 had no effect, reflected by the anti-Tau-1 staining in neurons (Figure 8B)¹³.

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**Figure 6.** TRPC3 is Highly Expressed in Human Ovarian Epithelial Tumors.

**Figure 7.** ATP Induced Current is Inhibited by the Anti-TRPC3 Antibody.

**Figure 8.** TRPC5 Channels Regulate Axon Formation.

A) E18 hippocampal neurons were electroporated before plating with control siRNA or siTRPC5 plus soluble EGFP. Neurons were then fixed at 48 hours following electroporation and stained with Anti-TRPC5 antibody and imaged. Representative images from control siRNA (top) and siTRPC5 (bottom) are shown in the left panel and the graphs quantifying the images are shown in the right panel. B) E18 neurons were electroporated before plating with control siRNA, siTRPC5, or siTRPC6. Neurons were fixed at 48 hours and evaluated for axon formation by staining with anti-Tau-1 antibody. Representative images are shown in the left panel while the corresponding quantification of polarized neurons (% of Control) and length (µm) in the neurons that did polarize is shown on the right. Scale bar: 20µm.

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Adapted from reference 14 with permission of The Society for Neuroscience.
TRPC6

Elevation in intracellular Ca\(^{2+}\) concentration can lead to changes in dendritic morphology. Tai et al. report that TRPC6 is highly expressed during the period of maximal dendritic growth (Figure 9) demonstrated in immunohistochemical studies using Anti-TRPC6 antibody\(^{15}\). The peak expression of TRPC6 in rat hippocampus was between postnatal day 7 and 14, a period known to be important for maximal dendritic growth. The authors show that TRPC6 promotes hippocampal neuron dendritic growth via the CaMKIV-CREB pathway\(^{15}\).

Boisseau et al. initiated a study in order to gain some insight about the tissue distribution of TRPCs\(^{16}\). The distribution of TRPC1, TRPC3 and TRPC6 throughout the cortical wall was studied using immunohistochemistry. The data indicate that TRPC1 is specifically found in the preplate located under the pial surface, using Alomone Labs’ Anti-TRPC1 antibody. Unlike TRPC1, both TRPC3 and TRPC6 were widely distributed throughout the cortical wall (using Anti-TRPC3 and Anti-TRPC6 antibodies). Furthermore, both channels were expressed by the same cortical cells.

Angiogenesis is important for development, tumor growth and metastasis. VEGF (vascular endothelial growth factor) plays a critical role in this process. Therefore, inhibiting the signaling pathway of VEGF has become an important objective in anticancer treatment. In the signaling pathway of VEGF has become an important objective in anticancer treatment. In the signaling pathway of VEGF, an intracellular increase in Ca\(^{2+}\) takes place through an intracellular Ca\(^{2+}\) release and extracellular Ca\(^{2+}\) entry.

TRPC6 is responsible for the extracellular entry of Ca\(^{2+}\) in human umbilical vein endothelial cells (HUVECs)\(^{17}\). Indeed, inhibition of TRPC6 by siRNA or by expression of a dominant negative form of the protein (protein levels subsequently verified by western blot analysis using Anti-TRPC6 antibody) suppressed HUVEC proliferation just as inhibition of the channel with a pharmacological inhibitor did. These data suggest that TRPC6 plays an important role for VEGF-induced angiogenesis and may be a target for potential cancer treatment\(^{17}\).

References:

# Related Products

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TRP Channel Modulators
- S-iodoresiniferatoxin: I-800
- 6'-iodoresiniferatoxin: I-805
- Resiniferatoxin: R-400

Intracellular Ca\(^{2+}\) Mobilizers
- Thapsigargin: T-650
- Ca\(^{2+}\) Ionophores
- Ionomycin: I-700
Ionotrophic ATP (P2X) Receptors

Alon Meir, Ph.D.

P2X receptors are surface membrane ligand-gated channel proteins that rapidly translate extracellular ATP elevation into an intracellular Ca$^{2+}$ surge which affects the cell’s secretion, gene expression, contraction and migration capabilities as well as viability and differentiation status. Here, we discuss recently published uses of Alomone Labs’ specific P2X antibodies in the growing field of ATP-gated channels.

Adenosine triphosphate (ATP) is an energy storing molecule in all cells. However, in several cellular systems, where it might be present in the extracellular space, ATP also serves as a neurotransmitter or hormone. Well studied examples of such systems include inflammation states, where the dead cell’s cytoplasmic content or “inflammatory soup” that contains a high level of ATP, affects lymphocytes and healthy cells. Another example is the extracellular space around synapses in the nervous system or around innervated tissue; where synaptic vesicles release neurotransmitters along with ATP (which exists at high concentrations) upon their fusion with the plasma membrane. In such cellular systems the sensing of extracellular ATP, which is translated from outside” signal.

The receptors for extracellular ATP are divided into metabotropic (P2Y) and ionotropic (P2X) receptors. P2Y receptors are G-Protein Coupled Receptors (GPCRs, of which 8 isoforms exist) and are not discussed further here.

P2X receptors are protein complexes, composed of three similar subunits that bear an ion channel within a membrane spanning domain, which gates open upon ATP binding (to an extracellular domain). All seven P2X channel isoforms (P2X1-7) are permeable to organic mono and divalent cations including Ca$^{2+}$ (for review on purinergic 7) are permeable to organic mono and divalent cations.

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The receptors for extracellular ATP are divided into metabotropic (P2Y) and ionotropic (P2X) receptors. P2Y receptors are G-Protein Coupled Receptors (GPCRs, of which 8 isoforms exist) and their downstream signaling is defined by the G proteins they are complexed with. They usually respond in a longer time range than P2X receptors and are not discussed further here.

P2X receptors are protein complexes, composed of three similar subunits that bear an ion channel within a membrane spanning domain, which gates open upon ATP binding (to an extracellular domain). All seven P2X channel isoforms (P2X1-7) are permeable to organic mono and divalent cations including Ca$^{2+}$ (for review on purinergic transmission, see reference 1).

The pharmacology of P2X receptor channels is the most common tool used for indicating and identifying the P2X subtype.

Alomone Labs specific P2X antibodies, besides having an important role in showing the localization of P2X channels, also assist in deciphering their physiological and pathological roles. We cite papers where these antibodies were used: Immunoprecipitation (IP) experiments to demonstrate physical interactions between P2X isoforms and with other proteins4; Western blot to demonstrate expression in cells or tissue extracts, or knockdown by siRNA or in knockout mice14,16; Immunohistochemistry (IH) and Immunocytochemistry (IC) experiments to localize the expression sites and patterns of the channels using different microscopic methods5,15,17,18,20.

Many studies explore the roles of P2X channels in white blood cells and in tissues affected by injury as well as in neurons and glia both in the context of stress and injury and in response to sympathetically released ATP.

Using Anti-P2X1 (#APR-001), Anti-P2X2 (#APR-003), Anti-P2X4 (#APR-002) and Anti-P2X7 (#APR-004) antibodies, P2X1, 2, 4 and 7 were detected in mouse urinary bladder, exemplifying the role and expression of these subunits in smooth muscle41. P2X2 was detected in pulmonary neuroepithelial bodies (probably forming a complex with P2X3)22.

P2X3 channel was found to be expressed in sensory neurons using Anti-P2X3 (#APR-016) antibody. Upon a decrease in Nerve Growth Factor (NGF) levels, these neurons are induced to express more P2X2/3 complexes, changing ATP derived signaling. In such sensory neurons, the expression of P2X3 was shown to depend on calcitonin gene related peptide41. Using Anti-P2X3 (#APR-016) antibody5. Upon a decrease in Nerve Growth Factor (NGF) levels, these neurons are induced to express more P2X2/3 complexes, changing ATP derived signaling. In such sensory neurons, the expression of P2X3 was shown to depend on calcitonin gene related peptide41. Using Anti-P2X4–/– mice. Scale bars: I, J, K, 20 µm; L, M, N, 50 µm.

**Figure 1. Expression of P2X4 Receptor Channel in Wild-type P2X4+/+ but not in Knock-out P2X4−/− Mice.**

A, C, E, Using Anti-P2X4 antibody (#APR-002), P2X4 subunit immunoreacts throughout the cell body layers of hippocampus and is absent in knock-out mice (B, D, F). Pyr, Pyramidal cell. G, H, Detection of β-galactosidase. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) staining in hippocampus of knock-out P2X4−/− mice but not in wild-type P2X4+/+ mice. Scale bars: A, B, G, H, 500 µm; C, D, 50 µm; E, F, 40 μ m. I, P2X4 subunit is immunoreactive in cerebellar Purkinje cells and is absent in the knock-out P2X4−/− mice (J). K, X-gal staining shows the expression of β-galactosidase in cerebellar Purkinje cells (P), as well as some cells of the granular layer (g) and mossy layer (ml) from knock-out P2X4−/− mice. L, P2X4 subunit immunoreactivity in acinar and ductal cells of the submandibular gland is completely absent in knock-out P2X4−/− mice (M). N, Extensive expression of β-galactosidase in submandibular gland of knock-out P2X4−/− mice. Scale bars: I, J, K, 20 µm; L, M, N, 50 µm.

Adapted from reference 17 with permission of The Society for Neuroscience.
P2X4 antibody, it was demonstrated that P2X4 is expressed in many tissues; the protein was detected in brain, lung and submandibular gland of wild type and absent in tissues from P2X4 knockout mice (Figure 1). In the CNS P2X4 was mainly in microglia and in hippocampal cells following ischemia (Figure 3). Morphine treatment enhances microglial migration by increasing the expression of P2X4. Expression of P2X4 and other P2X receptor channels were detected in neurons innervating the carotid body O2 chemoreceptors, suggesting a role in negative feedback loop that inhibits ATP releasing chemoreceptors during hypoxic stress. P2X4 and other P2X channels were also shown to be expressed in chicken mesenchymal cell cultures, suggesting a role in bone development and repair.

P2X4 was shown to heteromerize with P2X7 subunits by co-immunoprecipitation in an expression system. It was also shown for both P2X4 and P2X2 that mutating the ATP binding site causes reduced surface expression of the mutated subunit.

P2X7 receptor channel differs from all the other isoforms in that it is activated only by very high ATP concentration and in many cases P2X7 channels mediate a very robust intracellular Ca²⁺ elevation. This paper probably resolves a controversy regarding P2X7 antibody specific detection in knockout mice. In addition to the expression of physical and functional complex between the ATP receptor and the gap junction channel.

Although P2X7 is absent in macrophages of knockout mice, it was recently demonstrated, that functional channels are expressed in T lymphocytes of these mice. This paper probably resolves a controversy regarding P2X7 antibody specific detection in knockout mice. In addition to the expression of physical and functional complex between the ATP receptor and the gap junction channel.

Using Anti-P2X7 antibodies (#APR-004 and/or #APR-008, directed against intracellular and extracellular epitopes, respectively), showed that P2X7 channels are expressed in macrophages where they control IL-β release. Anti-P2X7 antibody was used to immunoprecipitate the channel from macrophages, later to be blotted with anti-Pannexin-1 antibody, suggesting a role in immune response.

Visualization of the P2X4 protein detected by using Anti-P2X4 antibody (#APR-002) in the L5 dorsal spinal cord by immunofluorescence analysis with confocal microscopy. Photographs show the P2X4 immunofluorescence in the dorsal horn 14 days after nerve injury (top), 14 days after sham operation (bottom left) and 7 days after the injection of CFA into the plantar surface of the hindpaw, an inflammatory pain model (bottom right). Scale bars, 200 µm. Adapted from reference 24 with permission of Nature Publishing Group.

**Figure 2. Marked Upregulation of P2X4 Levels in the Spinal Dorsal Horn Following Injury to the L5 Nerve.**

**Figure 3. in vivo Up-Regulation of P2X2,4 Proteins in Gerbil Hippocampus Following Ischemia.**

Nissl staining of the hippocampus of a sham-operated (A) or ischemic animal (B); P2X2 immunostaining (detected with Anti-P2X2 antibody (#APR-003)) of a sham-operated (C) or ischemic animal (D); P2X4 immunostaining (detected with Anti-P2X4 antibody (#APR-002)) of a sham-operated (E) or ischemic animal (F); arrows: CA1–CA2 transition zone. (G) Higher magnification of P2X2-immunolabeling of the CA1 region of an ischemic animal; arrows: network of fibers in the pyramidal cell layer and apical dendrites. (H) Higher magnification of P2X4-immunolabeling of the CA1 pyramidal cell layer of an ischemic animal; arrows: processes surrounding an unstained cellular body (marked by asterisks). (I) Higher magnification of P2X2-immunolabeling of the CA1 pyramidal cell layer of an ischemic animal. Meshwork of fibers and puncta surrounding unstained cellular bodies marked by asterisks. Scale BARS=100 µm (A–F); (G)=40 µm; (H, I)=10 µm. Adapted from reference 16 with permission of Elsevier.
Preparations of flexor digitorum brevis muscle and tibial nerve were fixed for either immunofluorescence, immunoelectron or confocal microscopy. Preparations of NMJs double labeled with antibodies against NF165 and SV2, visualised with FITC-conjugated secondary antibody (a, d and g), which labels motor axons and terminals, and P2X7 (using Anti-P2X7 antibody (APR-004)), visualised with a TRITC-conjugated secondary antibody (b, e and h), illustrates that P2X7RS appears to be localised to motor nerve terminal from birth (0 day old, a–c) through 4 (d–f) and 7 days old (g–i). Electron microscopy of a single nerve terminal bouton confirmed that immunoreactivity for P2X7RS was confined to the presynaptic nerve terminal bouton in adults (j) and was not found on terminal Schwann cells or postsynaptic muscle fibres. A single slice taken from a confocal stack through a portion of an NMJ, double labeled for P2X7RS endodomain (green, Anti-P2X7 antibody) and P2X7 ectodomain (red) antibodies (k). Extended orthogonal projections through the confocal stack (l and m) confirm co-localisation and suggests a small spatial differentiation between the endodomain (central) and the ectodomain (peripheral) labelling, as predicted from the intra- and extracellular domains of the receptor, which these antibodies were raised against. Scale bars: a–i = 10 µm, j = 500 nm, k = 5 µm.

Adapted from reference 20 with permission of Elsevier.
Staining of P2X3 in rat dorsal root ganglion (DRG) with **Anti-P2X3** antibody (#APR-016). Cells within the DRG were stained (see solid line frame enlarged in B) as well as fibers and the area of entry of dorsal root into spinal cord (see dashed line frame enlarged in C). The counterstain in B and C is DAPI, a fluorescent dye visualized in the UV range.

**Immunocytochemistry of P2X7.**

Immunocytochemistry of K562 living cells stained with **Anti-P2X7** (extracellular)-FITC antibody (#APR-008-F).

**Expression of P2X4 in Rat Brain.**

Immunohistochemical staining of P2X4 in rat brain reular nucleus using **Anti-P2X4** antibody (APR-002). A, P2X4 (green) appears in fibers surrounding cell shapes (arrows). B, Calbindin 28K (red) appears in large neurons. C, merge of P2X4 and Calbindin 28K suggest variable density of P2X4 expressing fibers on red nucleus neurons. DAPI is used as the counterstain (blue).

**Flow Cytometry Analysis of Intact living Jurkat T-cells.**

Western blot analysis of human platelets lysate:
1. Anti-P2X1 antibody (APR-001) (1:200).
2. Anti-P2X1 antibody, preincubated with the control peptide antigen.

**Related Products**

**Antibodies to Purinergic (P2X) Receptors**

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**References:**

Characterization of Neuronal K⁺ Channels

Phyllis Dan, Ph.D.

A wide variety of K⁺ currents recorded in neurons can be distinguished according to their functional and pharmacological properties. The identification of the channel family and subunits necessitates specific modulators and antibodies. Alomone Labs’ contribution to the advances made in K⁺ channels studies is quite broad. In this review, we summarize how some products have influenced advances made regarding the understanding of neuronal K⁺ channels.

K⁺ channels are tetrameric integral membrane proteins that form aqueous pores through which K⁺ can flow, and are found in many organisms. In neurons, K⁺ channels are involved in maintaining the resting potential, repolarizing the membrane after action potentials, and controlling the timing of repetitive firing.

One of the most useful reagents for investigating K⁺ channels is α-Dendrotoxin (RD-350), which specifically blocks Kv1.1 and Kv1.2 channels. Currents from mouse trigeminal ganglion neurons were divided into 3 classes, based on their firing properties. The sensitivity of the currents from each type of neuron to α-Dendrotoxin and rHeteropodatoxin-2 (RTH-340) (Figures 1A, 1B) made classification of the type of channel involved possible and enabled a mathematical model of the current to be built.

The loss of striatal dopamine in Parkinson’s disease model triggers a cell-type-specific reduction in the density of dendritic spines in D2 receptor-expressing striatopallidal medium spiny neurons. Kv1.2 channels regulate somatic, but not dendritic, excitability in these neurons as shown by their sensitivity to α-Dendrotoxin (Figure 2).

Degeneration of retinal ganglion cells (RGCs) is a major cause of visual impairment in optic neuropathies. The use of optic nerve transection which leads to the apoptotic death observed in these central nervous neurons has become an important tool to test potential neuroprotective approaches in vivo. It is well established that in some cellular systems, an increase in K⁺ currents, depletion of cytoplasmic K⁺ and cell shrinkage all lead to the activation of proteins involved in apoptosis and concomitantly to apoptosis itself. The link between these two events is still largely unknown. By inducing RGC degeneration by optic nerve transection, Koeberle et al. quite elegantly analyzed the involvement of various K⁺ channels in apoptosis in RGCs following axotomy using channel blockers and siRNA knockdown of each channel in vivo. In the retina from a healthy adult rat, Kv1.1 immunoreactivity was widespread using Anti-Kv1.1 antibody (#APC-009).
Figure 2. Kv1.2 Channels Regulate Somatic, but not Dendritic, Excitability in MSNs.

A) and B) Line scans taken 45–60 µm from the soma show that blockade of Kv1.2 channels with α-Dendrotoxin (0.5 µM) does not affect the amplitude of the bAP-evoked Ca2+ transient in dendrites (left traces in A and B) or adjacent spines (right traces in A and B) of either D1 or D2 MSNs (control: black, α-Dendrotoxin:red). Recordings were generated by injecting sequential depolarizing current steps at amplitudes just before and after rheobase potentials (100 and 125 pA, lower traces in A and B). Scale bar, 50µM in all main panels. i) In the normal retina, Fluorogold-labeled RGCs were retrograde transported to the somata by applying it to the freshly cut optic nerve stump. Anti-Kv1.1 and secondary antibody, conjugated to either Alexa 488 (green) or Cy3 (red). When used, Fluorogold (false-colored green) was retrograde transported to the somata of RGCs by applying it to the freshly cut optic nerve stump. Scale bar, 50µM in all main panels. 1. Kv1.1 immunoreactivity (green) is seen in the inner nuclear layer (INL); outer nuclear layer (ONL); and ganglion cell layer (GCL). Both RGCs and displaced amacrine cells were labeled with Kv1.1 antibody (arrows show examples). ii-iv) Control finelly luciferase-specific siRNA had no effect. Five days after Fluorogold (green), the control siRNA was applied to the freshly cut nerve stump, the Kv1.1 (red) staining pattern was similar to the naive retina, and most Fluorogold-labeled RGCs also stained Kv1.1 (yellow in merged image). Below the remaining micrographs, the boxed areas in the main images are color separated and shown at a higher magnification (scale bar, 20µM applies to all expanded images). v and vi) Kv1.1-specific siRNA depletes Kv1.1 in RGCs. Five days after axotomy and administration of Fluorogold and either of two siRNAs against Kv1.1, the channel staining (red) in RGCs decreased. B) Kv1.1 depletion reduces the death of RGCs. Summary of RGC densities in the inner, middle and outer eccentricities at 14 days after axotomy (**P < 0.001). Adapted from reference 5 with permission of Nature Publishing Group.

Figure 3. Expression Pattern of the Shaker-Family Kv1.1 Channel and Neuroprotection Following siRNA-Mediated Kv1.1 Depletion from Retinal Ganglion Cells (RGCs).

A) Kv1.1 immunoreactivity in the retina. Representative micrographs of transverse sections taken at the inner eccentricity and labeled with Anti-Kv1.1 antibody (APC-010) and secondary antibody, conjugated to either Alexa 488 (green) or Cy3 (red). When used, Fluorogold (false-colored green) was retrograde transported to the somata of RGCs by applying it to the freshly cut optic nerve stump. Scale bar, 50µM in all main panels. i) In the normal retina, Kv1.1 immunoreactivity (green) is seen in the inner nuclear layer (INL); outer nuclear layer (ONL); and ganglion cell layer (GCL). Both RGCs and displaced amacrine cells were labeled with Kv1.1 antibody (arrows show examples). ii-iv) Control finelly luciferase-specific siRNA had no effect. Five days after Fluorogold (green), the control siRNA was applied to the freshly cut nerve stump, the Kv1.1 (red) staining pattern was similar to the naive retina, and most Fluorogold-labeled RGCs also stained Kv1.1 (yellow in merged image). Below the remaining micrographs, the boxed areas in the main images are color separated and shown at a higher magnification (scale bar, 20µM applies to all expanded images). v and vi) Kv1.1-specific siRNAs deplete Kv1.1 in RGCs. Five days after axotomy and administration of Fluorogold and either of two siRNAs against Kv1.1, the channel staining (red) in RGCs decreased. B) Kv1.1 depletion reduces the death of RGCs. Summary of RGC densities in the inner, middle and outer eccentricities at 14 days after axotomy (**P < 0.001). Adapted from reference 5 with permission of Nature Publishing Group.
The neurons of the deep cerebellar nuclei (DCN) provide the last stage of cerebellar information processing by integrating an array of excitatory and inhibitory sensory motor input. To determine the distribution of ion channel types in large diameter deep cerebellar nucleus cells of the cerebellum, Anti-Kv3.1b antibody (#APC-014), Anti-Kv3.3 antibody (#APC-102), Anti-Kv1.1 antibody (#APC-107), Anti-Kv2.1 antibody (#APC-039) and Anti-Kv2.2 antibody (#APC-028) were used. These images (Figure 7) show that Kv3.1 and Kv3.3 immunolabel primarily over somatic membranes and the proximal 50 µm of dendrites of large diameter cells. Labeling was detected as a diffuse signal in the cytoplasmic region but also as a membrane-associated label that delineated the membranes of the soma and proximal dendrites.

Immunohistochemical Staining of Kv11.2 in Rat Hippocampus.

(A) Using Anti-Kv11.2 antibody (#APC-114), Kv11.2 appears as diffuse staining (green) that defines the boundary of the reticular nucleus of thalamus (arrow). (B) Staining with mouse anti-parvalbumin (PV, red) demonstrates both neuronal and diffuse staining. (C) Confocal merge of Kv11.2 and PV demonstrates overlap of the two markers labeling the reticular nucleus of the thalamus (orange).

Adapted from reference 6 with permission of The Society for Neuroscience.

Confocal projection of one MNTB neuron double-labeled for Kv3.2 using Anti-Kv3.2 antibody (#APC-120) and DAPI (blue nucleus) showing a highly stained putative initial segment region (arrow).

Adapted from reference 8 with permission of Blackwell Publishing Ltd.
Figure 7. Immunolocalization of Voltage- and Ca²⁺-Activated K⁺ Channels Involved in Spike Repolarization and Generation of AHPs.

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In each case, ion channels (left column, Cy3) are colocalized with MAP-2 (right column, Alexa-488) as a general structural counterlabel. A) and B) Immunolabel for Kv3.1 and Kv3.3 subtypes using Anti-Kv3.1 and Anti-Kv3.3 antibody (APC-015) of high-threshold voltage-activated K⁺ channels are located on the soma and proximal dendrites of large diameter DCN neurons. C) Immunolabel for Kv3.1 (BK) K⁺ channels using Anti-Kv3.1 antibody (APC-015) can be visualized as a punctate label on the membranes of MAP-2–labeled DCN neurons. D) and E) DCN cells labeled for 2 of the SK K⁺ channel isoforms Kv2.1 (SK1) using Anti-Kv2.1 antibody (APC-039) and Kv2.2 (SK2) using Anti-Kv2.2 antibody (APC-028). Scale bars 20µm.

Adapted from reference 9 with permission of The American Physiological Society.

References

Voltage-Gated \(Ca^{2+}\) Channels in the Cardiovascular System

Etai Shpigel, Ph.D., Yossi Anis, Ph.D.

Voltage-gated \(Ca^{2+}\) channels play a pivotal role in the regulation of a wide range of cellular processes, including membrane excitability, \(Ca^{2+}\) homeostasis, protein phosphorylation, gene regulation, muscle contraction and vesicular secretion. The L-type voltage-gated \(Ca^{2+}\) channels are the most common. This brief review aims at demonstrating advances made in research regarding voltage-gated \(Ca^{2+}\) channels in the cardiovascular system and the contributions of Alomone Labs in the field.

Voltage-Gated \(Ca^{2+}\) Channels in the Cardiovascular System

In cardiac and smooth muscle cells the predominant voltage-gated \(Ca^{2+}\) channels (CaV) are the dihydropyridine-sensitive CaV1.2 (\(\alpha_{1C}\)) channels (also called dihydropyridine receptor or L-type channel). These channels mediate long-lasting \(Ca^{2+}\) currents as a critical step in excitation-contraction coupling and have a central role in controlling cardiac function\(^2,3\). The CaV1.2 channels provide a trigger for intracellular \(Ca^{2+}\) release during excitation-contraction coupling in a process known as \(Ca^{2+}\)-induced \(Ca^{2+}\) release. Dysregulation of CaV\(_{1.2}\) channels in cardiac muscles leads to \(Ca^{2+}\) overload that influences the plateau phase of the cardiac action potential, and disrupts pacemaker activity in the sinoatrial node. The \(\alpha_1\) subunit (the pore forming subunit) is a target for calcium channel blockers such as dihydropyridines (DHPs), phenylalkylamines, and benzothiazepines, which are widely used for treating hypertension, angina pectoris, and cardiac arrhythmias\(^4,5\).

In the cardiac muscle, a distinct \(\alpha_1\) subunit (\(\alpha_{1.2a}\) cardiac isoform), an \(\alpha_2\delta\) subunit, and several isoforms of \(\beta\) subunits (\(\beta_{1\alpha}\) and \(\beta_{2\alpha}\)) have been identified and implicated to form the CaV1.2 channel. The CaV1.2a \(\alpha_{1C}\) subunit is an alternative splice variant of exon 1 which differs from the vascular smooth muscle isoform at the N-terminus.

Two forms of the CaV1.2a \(\alpha_{1C}\) subunit (\(\approx 240\) and \(210\) kDa) are expressed in the cardiac muscle which differ in a truncation at the C-terminus. Whereas the majority of \(\alpha_{1C}\) subunits isolated from cardiac muscle are truncated (demonstrated using Anti-CaV1.2a antibody, (\#ACC-013))\(^6\), the cleaved distal C-terminus remains associated with the truncated \(\alpha_1\) subunit of CaV1.2a following proteolytic processing, and peptides derived from it can control the channel’s activity. The cardiac CaV1.2a activity is highly regulated by a variety of stimulus. The C-terminal region is a target site for \(\beta\)-Adrenergic regulation and for phosphorylation by PKA\(^7\). Stimulation of cardiac cells by hLIF (human Leukemia Inhibitory Factor) (\#L-200), enhances CaV1.2 currents through phosphorylation by ERK at S1829 of the \(\alpha_{1C}\) subunit\(^7\).

Although physiologically essential for normal cardiac function, increased level of CaV1.2 channels or activity have been implicated in pathological processes involving \(Ca^{2+}\) disturbance, including cardiac ischemia and myocardial stunning\(^8\). The expression level of CaV1.2 in the heart was demonstrated using Anti-CaV1.2 antibody (\#ACC-03) and its expression was up-regulated when PI3K and \(Ca^{2+}/calmodulin\)-dependent protein kinase II (CamK II) were overexpressed\(^9,10\) (Figure 1).

In hypertension diseases, the level of vascular voltage-gated L-type calcium channel currents and vascular tone are increased, as a result of the CaV1.2 non-cardiac form overexpression (demonstrated in different rodent models of hypertension using Anti-CaV1.2 antibody\(^9\)).

The physiological importance of the \(\beta3\) subunit...
in the cardiovascular system was investigated using gene targeted mutant mice. In this system the lack of β3 subunit in β3-null mice was confirmed using Anti-CaV β3 antibody (#ACC-008). Western blot analysis using Anti-CaV1.2a antibody demonstrated a remarkable reduction in the membrane expression of αo subunit in these mice. The reduction of αo was accompanied by a significant decrease in Ca2+ current density, a slower inactivation rate of Ca2+ channel, and reduced dihydropyridine-sensitivity11.

Ca1.3 channels play a pivotal role in the generation of cardiac pacemaker activity by contributing to diastolic depolarization in the sinoatrial node (SAN). Abnormal Ca1.3 trafficking caused by ankyrin-B dysfunction leads to abnormal sinoatrial node (SAN) excitability and causes SAN disease. In this syndrome the cardiac distribution of Ca1.3 was demonstrated by immunocytochemistry staining using the Anti-CaV1.3 antibody (#ACC-005) (Figure 2)13.

Ca2+ currents, through voltage-gated Ca2+ channels play a key role during excitation-contraction coupling in smooth muscle cells (SMCs) of the vascular system. Using vascular smooth muscles cells, L-, P- and Q-type voltage-gated Ca2+ channels were found to be expressed using Anti-CaV2.1 antibody (#ACC-001) as well as Anti-CaV1.2 antibody. Additional information about the identity of these channels was confirmed using Calciseptine (RC-500) known to inhibit the L-Type Ca2+ channels and ω-Agatoxin IVA (RA-500) which inhibits P and Q-type Ca2+ channels14. Figure 3 shows smooth muscle cells treated with Calciseptine, showing that L-type Ca2+ channels are indeed involved Ca2+ currents in these cells15.

**Figure 2.** The Expression of Ca1.3 but not Ca1.2 or Ca3.1 Channels in Sinoatrial Nod (SAN) Cell Membranes is Dependent on Ankyrin B Levels.

**Figure 3.** Calciseptine Inhibits L-Type Ca2+ Currents in Smooth Muscle.
Expression of Cav1.2a in Rat Heart.

Western blot analysis of rat heart membranes:
1. Anti-Cav1.2a antibody (Acc-013) (1:200).
2. Anti-Cav1.2a antibody, preincubated with the control antigen.

Immunohistochemical staining of Cav1.2a in rat heart using Alomone rabbit Anti-Cav1.2a antibody (Acc-013). Cav1.2a was visualized with immuno-peroxidase methods and final brown-black diaminobenzidine color product (arrows in A). The counterstain is cresyl violet. When the antibody was pre-incubated with the immunogen, staining was blocked (B).

FS-2 Inhibits L-Type Ca\(_2\) Channels Expressed in Xenopus Oocytes.

Left: 500 nM FS-2 (f1700) blocked 50% of L-type Ca\(_2\) channels (Cav1.2, β3) in Xenopus oocytes across the voltage range. Right: Example traces of Ca\(^{2+}\) current responses to depolarization to +20 mV before and during application of the toxin.
Electrophysiological Detection of Ca\textsuperscript{2+} Currents Induced by (-)-Bay K 8644.

The effect of 5 µM (-)-Bay K 8644 (B-350) on heterologously expressed L-type Ca\textsuperscript{2+} currents (Ca\textsubscript{v}1.2 /α\textsubscript{2}δ1 / β2a, RNA injected to Xenopus oocytes). Left: I-V relation before (squares) and during (circles), bath perfusion of the compound. Right: An example of current response to 200 ms depolarization to +20mV (from holding potential of -100 mV) before (black) and during (green) perfusion of the drug.

Calcicludine Inhibits L-Type Ca\textsuperscript{2+} Channels Heterologously Expressed in Xenopus Oocytes.

Left: 0.5 µM Calcicludine (C-650) blocked 50% of L-type Ca\textsubscript{v} channels (Ca\textsubscript{v}1.2, β3) in Xenopus oocytes across the voltage range. Right: Traces of Ca\textsuperscript{2+} current responses to depolarization to +20 mV before and during application of the toxin.

Western blot analysis of rat heart membranes:
1. Anti-human-Ca\textsubscript{v}1.2 antibody (RACK-022) (1:200).
2. Anti-human-Ca\textsubscript{v}1.2 antibody, preincubated with the control peptide.

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Voltage-Gated Ca\textsuperscript{2+} Channel Activator
(-)-Bay K8644 | B-350 |
α-Latrotoxin: A Potent Inducer of Secretion

Alon Meir, Ph.D.

α-Latrotoxin is a 130 kD protein toxin that is found in the black widow spider venom and is the only protein in the venom that affects mammals. α-Latrotoxin affects the nervous system of the bitten organism by causing massive transmitter release in many synapses, emptying the signaling reservoir at once which paralyzes the prey. Owing to these characteristics and to the vast knowledge that has been accumulated regarding its mode of action, Alomone Labs’ α-Latrotoxin is an effective molecular tool in the study of secretion, both in synapses and in endocrine systems as is reviewed in this brief report.

Mechanism of Action

α-Latrotoxin binds to two types of receptors on presynaptic nerve endings: Neurexin and Latrophillin (CIRL). These interactions facilitate the formation of α-Latrotoxin tetramers which function as cation-selective pores in membranes. Both receptors are essential for the toxin pore formation mechanism (membrane integration) but not for the toxin induced exocytosis. The binding of α-Latrotoxin to neurexin is Ca²⁺-dependent while its binding to CIRL is Ca²⁺-independent. Cation-selective pores formed by α-Latrotoxin can induce release by allowing Ca²⁺ to enter into the terminal, forming a Ca²⁺-dependent release pathway (which is essential for triggering the release of monoamines or peptides from synapses). However, in synapses transmitting GABA, glutamate or Ach, release also occurs in Ca²⁺ free conditions. This is probably achieved in addition by direct interaction of α-Latrotoxin with synaptic proteins, which bypasses the Ca²⁺ binding event and leads to activation of exocytosis.

Experimental Uses of α-Latrotoxin

α-Latrotoxin, at sub-nanomolar and nanomolar concentrations, induces secretion in several types of cellular systems. Its effect was examined in CNS and NMJ preparations, where the toxin causes neurons to release neurotransmitters as well as in other secreting (mainly endocrine) systems where the toxin induces hormone secretion. Therefore, α-Latrotoxin is a very efficient molecular tool to be used in studies of these processes.

Alomone Labs’ α-Latrotoxin (#LSP-130) (0.3-1 nM) was used to influence smooth muscle contraction or relaxation, by causing transmitter release from enteric as well as sensory neurons that innervate such muscles. Transmitter release was also monitored directly in sympathetic or DRG neurons and the influences of 1-3 nM toxin was examined (Figures 1,2,3,4).

In motor nerve-muscle preparations α-Latrotoxin was used (2.5-10nM) to mimic neurotransmitter release while examining its influence on autoimmune inflicted diseases, synaptic proteins deletions, pharmacological agents and other pathologic or physiologic conditions.

In the central nervous system (CNS), α-Latrotoxin is also a very potent inducer of secretion and therefore is widely used as a tool to study the regulation of synaptic transmission. It was used in vivo (0.5-3 nM) to stimulate secretion, and later to monitor the presence of β-Amiloide peptides in brain fluids or neurotransmitter levels following synaptic activation (Figure 5).

In studies of the various functions of central synapses such as synaptic inhibition, presynaptic inhibition, synaptic machinery and growth cone physiology, α-Latrotoxin is used at very low concentrations (0.5-10nM) to induce secretion. Particularly, in the hippocampus (synaptosomes, tissue culture and slices), several groups have studied the effect of continuous transmitter release, induced by α-Latrotoxin, on the biochemistry and biophysics of synapses (Figure 6).

α-Latrotoxin is shown to be an effective inducer of secretion in several other cell types such as PC-12, P-19, AT201, chromaffin cells, hypophysal cells, glia and pancreatic β-cells (Figures 7,8). In PC-12 cells, many aspects of the need and function of proteins involved in the release machinery were investigated using α-Latrotoxin as a tool to activate secretion. In pancreatic β-cells, the action of the toxin and its effect on different processes involved in hormone secretion was characterized.
Figure 1. α-Latrotoxin Fails to Increase Recycling Vesicle Pool Size or the Number of Vesicles Released in DTB-Treated Mice.

A) and B) Representative images showing the time course of FM1-43 destaining triggered by bath application of 2 µg/ml α-Latrotoxin (#LSP-130) in an untreated terminal and a dim terminal from a DTB-treated mouse. The destaining in untreated terminals appeared to be uniform throughout a terminal and was almost completed in 60 min. In DTB-treated dim terminals, α-Latrotoxin caused redistribution of FM1-43 fluorescence within the nerve terminals, but did not cause dye destaining. C) The average intensity of fluorescence measured before and at 20 and 60 min after α-Latrotoxin application. The data are presented as the intensity normalized to the average fluorescence values prior to α-Latrotoxin treatment (control). The data are the means ± SE of 4 untreated and 5 DTB-treated mice, respectively. *, a value significantly different from untreated control (P ≤ 0.01).

Adapted from reference 19 with permission of The American Physiological Society.

Figure 2. Increased Electrochemical Activity in Sympathetic Nerve Terminals in Rat Mesenteric Arteries Following α-Latrotoxin Treatment.

Adapted from reference 3 with permission of Elsevier.

Figure 3. Enhanced Recording of Spontaneous Contractions of the Circular Muscle of the Guinea-Pig Distal Colon Circular Muscle in Response to α-Latrotoxin.

Strips of circular muscle and attached myenteric plexus were mounted in organ baths and circular muscle contraction was measured with isometric transducers. A number of drugs was added to block transmission from all but the excitatory motor neurons. Atropine was also added to isolate the non-cholinergic component of transmission. In these conditions, α-Latrotoxin (#LSP-130) produced an increase in the amplitude of spontaneous contractions after a delay of 10 min.

Adapted from reference 6 with permission of Blackwell Publishing Ltd.
Acute brain slices were made from 4- to 5-week-old wild-type (B6/SJL) or Tg2576 mice. A) To determine the effect of synaptic vesicle exocytosis on extracellular β-Amyloid peptide levels in the absence of synaptic activity, Tg2576 brain slices were cultured for 2 hours in the presence of 0.5 nM α-Latrotoxin and/or a cocktail of activity inhibitors including 100 nM TTX, 10 mM NBQX, and 50 mM APV. α-Latrotoxin alone caused a 35% ± 6.9% increase in β-Amyloid peptide levels, whereas the inhibitor cocktail lowered β-Amyloid peptide levels by 18.0% ± 4.1% compared to untreated slices. α-Latrotoxin plus the inhibitor cocktail resulted in 13.3% more extracellular β-Amyloid peptide as compared to untreated slices and 38.3% ± 6.2% more β-Amyloid peptide compared to the inhibitor cocktail alone (n = 12–15 per group). At the end of each experiment, brain slices were washed with PBS and processed by Western blot analysis for the presence of APP-CTF. B) Neither the level of α-CTF nor the level of β-CTF changed over the 2 hours culture period. C) Representative lanes from Western blot. Data represent mean ± SEM. Adapted from reference 29 with permission of Elsevier.

Figure 4. Ultrastructural Features of Control and α-Latrotoxin Treated Neuromuscular Junctions.

Figure 5. Extracellular β-Amyloid Peptide Levels Are Directly Linked to Synaptic Vesicle Exocytosis.
Figure 6. α-Latrotoxin Reduces a Rise of [Ca\textsuperscript{2+}]\textsubscript{i} in Cultured Rat Hippocampal Neurons.

A) Four examples of α-Latrotoxin (LSP-130) effect on [Ca\textsuperscript{2+}]\textsubscript{i} at indicated toxin concentrations. [Ca\textsuperscript{2+}]\textsubscript{i} was assayed by the ratio of fura-2 fluorescence with alternating excitation at 340 and 380 nm. Note that as the concentration of α-Latrotoxin increases, so does the increment in [Ca\textsuperscript{2+}]\textsubscript{i}. α-Latrotoxin was perfused throughout the experiment starting from the times indicated by arrows. B) Dose dependence of α-Latrotoxin’s effect on the [Ca\textsuperscript{2+}]\textsubscript{i} increase (∆[Ca\textsuperscript{2+}]\textsubscript{i}). Data were averaged from five to nine cells. The maximal effect of α-Latrotoxin is achieved at 3 nM.

Adapted from reference 71 with permission of Blackwell Publishing Ltd.

Figure 7. α-Latrotoxin Induces [Ca\textsuperscript{2+}]\textsubscript{i} Elevation in the Presence of Extracellular Ca\textsuperscript{2+} in β-Cells.

Figure 8. Analysis of Recombinant α-Latrotoxin.

A) Analysis of purified recombinant α-Latrotoxin. Recombinant α-Latrotoxin along with native α-Latrotoxin (LSP-130) were analyzed by SDS-PAGE and Coomassie staining. B) GST-cleaved recombinant α-Latrotoxin stimulates Ca\textsuperscript{2+}-dependent exocytosis with a similar potency to native α-Latrotoxin. A stimulation of NE secretion from PC12 cells by indicated concentrations of native α-Latrotoxin (white squares; n=12), cleaved recombinant α-Latrotoxin (black squares; n=10), and GST-α-Latrotoxin (gray squares; n=3) is shown. Error bars indicate SEM.

Adapted from reference 57 with permission of The Society for Neuroscience.
References


Corrigendum

In Modulator issue No. 22 Fall 2008, Alomone Labs published a review titled “K Channels in Cardiomyocytes”. Figure 1 should have been acknowledged to Dr. S.A. Jones of the University of Hull and not as published. Alomone Labs regrets this error.

In Modulator issue No. 22 Fall 2008, Alomone Labs published a review titled “The involvement of Ion Channels in Cell Proliferation”, Reference 2 in the text should have been issued to Vincente, R. et al. (2003) / Biol. Chem. 278(47), 46707 and not as published. Alomone Labs regrets any inconvenience that this error may have caused.
# Fluorescently Labeled Antibodies

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Alomone Labs is pleased to offer a new line of its well-characterized antibodies directly conjugated to a new generation of specially developed, bright fluorescent dyes by ATTO-TEC.

ATTO dyes are well known in the field of fluorescent technology and are characterized by strong absorption (high extinction coefficient), high fluorescence quantum yield, and high photo-stability. ATTO dyes are analogous to the established Alexa dyes and were found to be comparable to any fluorescent technology in the market.

The proven quality of the Alomone antibodies together with the bright ATTO dyes generate an invaluable tool that undergoes careful quality control and is specially suited for applications that require simultaneous labeling of different markers.

Alomone Labs is currently offering primary antibodies directly conjugated to the following ATTO dyes: ATTO-488 (green) and ATTO-550 (orange).

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<tr>
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<td>Orange</td>
<td>λex 554 nm; λem 576 nm in 0.1 M phosphate pH 7.0</td>
<td>TAMRA, Cy3, Alexa-555</td>
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**Anti-Angiotensin II Receptor Type-2 (AT_2 Receptor) (extracellular)-ATTO-488**

*Product#: AAR-012-AG*

Expression of AT_2 Receptor in mouse 3T3-L1 cells

Expression of AT_2 Receptor in rat brain

Immunohistochemical staining of intact live mouse 3T3-L1 cells with Anti-AT_2 Receptor (extracellular)-ATTO-488 antibody (AAR-012-AG) (1:20). Live view of the same field was superimposed to the fluorescent one.

Immunohistochemical staining of AT_2 receptor in rat brain. Rat frozen free floating sections were stained with Anti-AT_2 Receptor (extracellular)-ATTO-488 antibody (AAR-012-AG) (1:20). The AT_2 receptor (green) was detected in neurons in the vicinity of the hypothalamic paraventricular nucleus (PVN). In some neurons (thick white arrow), axonal processes with varicosities were observed (thin white arrow). Nuclei were visualized with DAPI counterstain (blue).
**Anti-Ca1.2-ATTO-488**

*Product#: ACC-003-AG*

Expression of Ca1.2 channel in rat pancreas

Immunocytochemical staining of MS1 cells using Anti-Ca1.2-ATTO-488 antibody (1:50) (green). A, Intracellular staining of Paraformaldehyde-fixed and permeabilized MS1 cells. B, The cell-permeable dye Hoechst 33342 (blue) was used for nuclear staining. C, Merged images of panels A and B.

Expression of Ca1.2 channel in mouse pancreatic microvascular endothelial cells (MS1)

**Anti-Kir4.1-ATTO-488**

*Product #: APC-035-AG*

Immunohistochemical staining of frozen sections of rat cerebellum using Anti-Kir4.1-ATTO-488 antibody (1:50). Staining is specific for Bergmann glial cell processes (white arrows) in the molecular layer (ML) and astrocytes (yellow arrows) in the granular layer (GL). Purkinje cell bodies are stained with fluorescent Nissl stain (red). Hoechst 33342 (blue) is used as counterstain.

**Anti-AMPA Receptor 2 (GluR2) (extracellular)-ATTO-488**

*Product #: AGC-005-AG*

Expression of GluR2 in mouse cerebellum

Distribution of GluR2 in the molecular layer of the mouse cerebellum. Frozen free-floating sections were stained with Anti-AMPA Receptor 2 (GluR2) (extracellular)-ATTO-488 antibody (1:20). Both dendrites of Purkinje cells (horizontal arrows) were stained (green). DAPI counterstain (blue) helps define the layers: granule (G), Purkinje (P), and molecular (M).
Anti-Angiotensin II Receptor Type-1 (AT₁ Receptor) (extracellular)-ATTO-550

Product#: AAR-011-AO

Expression of AT₁ Receptor in rat C6 glioma cells

Immunohistochemical staining of AT₁ receptor in rat kidney. Paraffin embedded section of rat kidney showing the most inner layer of the cortex. Notice that intense stain is present in proximal tubes (P) but not in collecting ducts (C) in the cortical labyrinths (CL). Also note that no staining is present both in thin portions of the Loop of Henle or in the collecting ducts in the medullar rays (MR). Slides were treated with citrate for antigen retrieval and then incubated overnight at 4°C with Anti-AT₁ Receptor (extracellular)-ATTO-550 antibody (AAR-011-AO) (1:50) (Red). Nuclei are visualized with Hoechst 33342 (blue).

Immunocytochemical staining of live intact rat C6 glioma cells with Anti-AT₁ receptor (extracellular)-ATTO-550 antibody (AAR-011-AO) (A). Live view of the same field (B).

Anti-Human p75NTR (extracellular)-ATTO-550

Product#: ANT-007-AO

Expression of p75NTR in rat dorsal root ganglion (DRG) cells

Surface expression of p75NTR in rat dorsal root ganglion (DRG) cells. Intact live DRG neurons were labeled with Anti-mGluR1 (extracellular) antibody (KAGC-006) (1:100) followed by goat anti-rabbit Alexa 488 secondary antibody (green). The cells were then labeled with Anti-Human p75NTR (extracellular) ATTO-550 antibody (ANT-007-AO) (1:50) (red). Nuclei were visualized using the cell-permeable DNA binding dye Hoechst 33342 (blue). Note that the Anti-Human p75NTR (extracellular) ATTO-550 antibody does not stain all DRG neurons as expected. Colocalization is observed in some nerve fibers of the mGluR1 and p75NTR receptors.

Expression of p75NTR in p75NTR-transfected 3T3 cells

Immunocytochemical staining of p75NTR in p75NTR-transfected 3T3 cells. (A) Staining of intact live p75NTR-transfected 3T3 cells with Anti-Human p75NTR (extracellular)-ATTO-550 antibody (ANT-007-AO) (1:100). (B) Live view of the same field as in (A).
Anti-Aquaporin 2-ATTO-550

Expression of Aquaporin 2 in rat kidney

Immunohistochemical staining of paraffin embedded region of rat kidney showing a transversal cut of the inner medulla near to the renal papilla. Aquaporin 2 is detected in collecting ducts but not in thin segments of the loop of Henle. Slides were treated with citrate for antigen retrieval and then incubated overnight at 4°C with Anti-Aquaporin 2-ATTO-550 antibody (AQP-002-AO) (1:50) (Red). Nuclei are visualized with Hoechst 33342 (blue).

Anti-STIM1 (extracellular)-ATTO-550

Expression of STIM1 in rat pancreas

B. Hoechst 33342 (blue) is used as the counterstain.
C. Merged images of panels A and B.

Expression of STIM1 in rat basophilic leukemia cells (RBL)

Immunocytochemical staining of STIM1 in live RBL cells
A. Extracellular staining of cells with Anti-STIM1 (extracellular)-ATTO-550 antibody (ACC-063-AO) (1:20) (red).
B. Nuclear staining of cells using the cell-permeable dye Hoechst 33342.
C. Merged images of panels A and B.
Immunohistochemical staining of P2X7 in rat paraffin embedded endocrine and exocrine pancreas sections using Anti-P2X7-ATTO-550 antibody (APR-004-AO) (1:20) (red). Staining is highly specific for endocrine cells of the Isle of Langerhans. Counterstain is Hoechst 33342 (blue).

Expression of P2X7 in rat pancreas

Perfusion fixed, frozen free-floating mouse brain sections were stained with Anti-Kv1.5-ATTO-550 (APC-004-AO) (1:50) (red). Staining was detected in cerebellar Bergmann glial cells (white arrows). The blue (DAPI) is a counterstain visualizing nuclei of all cells. G = granule layer, P = Purkinje layer, M = molecular layer.

Kv1.5 expression in mouse cerebellum

Immunocytochemical staining of α₁B-Adrenoceptor in living GH3 cells

Perfusate extracellular staining of cells with Anti-α₁B-Adrenoceptor-ATTO-488 (extracellular) antibody (AAR-018-AG) (1:100) (green). Nuclear staining of cells using the cell-permeable dye Hoechst 33342 (blue).

Anti-α₁B-Adrenoceptor-ATTO-488 (extracellular)
Flow cytometry is a technique for counting, examining, and sorting microscopic particles suspended in a stream of fluid. It allows simultaneous multiparametric analysis of the physical and/or chemical characteristics of single cells flowing through an optical and/or electronic detection apparatus. The flow cytometer was developed in the 1970’s and rapidly became an essential instrument for biological studies. Extracellular antibodies directly labeled with fluorescein are an important tool for this application.

**Anti-Kv1.3 (extracellular)-FITC**

*Product#: APC-101-F*

Flow cytometry analysis of intact living Jurkat T-cells:
- Unstained cells.
- Cells + Anti-Kv1.3 (extracellular)-FITC antibody (#APC-101-F).

**Anti-Kv11.1 (HERG) (extracellular)-FITC**

*Product#: APC-109-F*

Flow cytometry analysis of intact living K562 (human chronic myelogenous leukemia) cells (A) and Jurkat (human T cell leukemia) cells (B):
- Unstained cells.

**Anti-P2X7 (extracellular)-FITC**

*Product#: APR-008-F*

Flow cytometry analysis of intact living Jurkat T-cells:
- Unstained cells.
- Cells + Anti-P2X7 (extracellular)-FITC antibody (#APR-008-F).
Anti-Proteinase-Activated Receptor-4 (extracellular)-FITC

**Product #: APR-034-F**

Flow cytometry analysis of PAR-4 expression in live intact HL-60 (human promyelocytic leukemia) cell line:
- Unstained HL-60 cells.
- HL-60 cells + Anti-Proteinase-Activated Receptor-4 (extracellular)-FITC antibody (APR-034-F).

Anti-Human Orai1 (extracellular)-FITC

**Product #: ACC-060-F**

Flow cytometry analysis of live Jurkat (human T cell leukemia) cells:
- Unstained cells.
- Cells + Anti-Human Orai1 (extracellular)-FITC antibody (ACC-060-F).

Anti-Human p75<sup>NTR</sup>-FITC

**Product #: ANT-007-F**

Host: Rabbit (Polyclonal).
Reactivity Confirmed: R, H

Flow cytometry analysis of live intact rat glioma C6 cells:
- Unstained cells.
- Cells + Anti-Human p75<sup>NTR</sup>-FITC antibody (ANT-007-F).

Anti-Rat p75<sup>NTR</sup>-FITC

**Product #: AN-170-F**

Host: Mouse (Monoclonal).
Reactivity Confirmed: Rat only

Flow cytometry analysis of PC12 cells:
- 1 µl per 1x10<sup>6</sup> cells (1:50 dilution).
- Unstained cells.
- Cells + Anti-Rat p75<sup>NTR</sup>-FITC antibody (AN-170-F).
**Anti-TRPV2 (extracellular)-FITC**

*Product #: ACC-039-F*

Flow cytometry analysis of intact living RBL cells:
- Unstained cells.
- Cells + Anti-TRPV2 (extracellular)-FITC antibody (ACC-039-F).

**Anti-α₁B-Adrenoceptor-ATTO-488 (extracellular)**

*Product #: AAR-018-AG*

Flow cytometry analysis of intact living GH3 cells:
- Unstained cells.
- Cells + Anti-α₁B-Adrenoceptor-ATTO-488 (extracellular) antibody (AAR-018-AG) (10µg/5x10⁵ cells).
**Anti-Kv1.5-ATTO-550**

**Product #: APC-004-AO**

Sizes: 50 µl
Host: Rabbit.
Epitope: GST fusion protein with sequence corresponding to residues 513-602 of mouse Kv1.5 (Accession Q61762).
Applications: IH

**Kv1.5 expression in rat cerebellum**

Perfusion fixed, frozen free-floating rat brain sections were stained with Anti-Kv1.5-ATTO-550 (APC-004-AO) (1:100) (red). Staining was detected in cerebellar Bergmann glial cells (white arrows). The blue (DAPI) is a counterstain visualizing nuclei of all cells. Abbreviations: G = granule layer, P = Purkinje layer, M = molecular layer.

**Western blot analysis of Kv1.5**

Western blot analysis of mouse brain (lanes 1 and 3) and kidney (lanes 2 and 4) membranes:
1, 2. Anti-Kv1.5 antibody (APC-123), (1:200).
3, 4. Anti-Kv1.5, preincubated with the control peptide antigen.

---

**Anti-Kir4.1-ATTO-488**

**Product #: APC-035-AG**

Sizes: 50 µl
Host: Rabbit.
Epitope: Peptide (C)KLEESLREQAEKEGSALSVR, corresponding to residues 356-375 of rat Kir4.1 (Accession P49655).
Applications: IH

Immunohistochemical staining of frozen sections of rat cerebellum using Anti-Kir4.1-ATTO-488 antibody (APC-035-AG) (green) (1:50). Staining is specific for Bergmann glial cells prolongations (white arrows) in the molecular layer (ML) and astrocytes (yellow arrows) in the granular layer (GL). Purkinje cell bodies are stained with fluorescent Nissl stain (red). Hoechst 33342 (blue) is used as counterstain.

**Western blot analysis of Kir4.1**

Western blot analysis of TRPV1 in rat DRG:
1. Anti-Rat TRPV1 (extracellular) antibody (ACC-029), (1:100) followed by Goat anti-rabbit-ATTO-594 secondary antibody (red). Counterstain is Hoechst 33342 (blue). TRPV1 is expressed in medium and small DRG.

---

**Anti-Rat TRPV1 (extracellular)**

**Product #: ACC-029**

Sizes: 50 µl
Host: Rabbit.
Epitope: Peptide (C)NSLPMESTPHK*SRGS corresponding to amino acid residues 605-619 of rat TRPV1 with replacement of cysteine 616 (C616) with serine (*S) (Accession O35433).
Applications: WB, IH

Immunohistochemical staining of rat DRG neurons using Anti-Rat TRPV1 (extracellular) antibody (ACC-029), (1:100) followed by Goat anti-rabbit-ATTO-594 secondary antibody (red). Counterstain is Hoechst 33342 (blue). TRPV1 is expressed in medium and small DRG.

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**Expression of Kir5.1 in rat kidney**

Immunohistochemical staining of paraffin embedded sections of rat kidney using Anti-Kir5.1 antibody (#APC-123) (1:100). Kir5.1 (brown staining) is expressed in both proximal tubules (PT) and distal tubules (DT) in the renal cortex. Note that collecting ducts (CD) are less stained while glomeruli (G) are negative. Counterstain is Hematoxilin.

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**New Products**

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### Antibodies to G-Protein Coupled Receptor

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### Anti-D3 Dopamine Receptor (extracellular)

Product #: ADR-003

**Sizes:** 50 µl | 0.2 ml  
**Host:** Rabbit  
**Epitope:** Peptide CGAENSTGVNRARPH corresponding to amino acid residues 15-29 of rat D3 Dopamine receptor (Accession P19020).  
**Applications:** WB, IHC

**Expression of D3 Dopamine Receptor in rat striatum**

- A. Perfusion-fixed frozen rat brain sections were stained with Anti-D3 Dopamine Receptor (extracellular) antibody (ADR-003) (1:100).  
- A. D3 Dopamine receptor appears in a subset of striatal neurons (green fluorescence).  
- B. The same section was stained for parvalbumin (red fluorescence), a marker of interneurons.  
- C. Merging the two images demonstrates that D3 Dopamine receptor localization includes parvalbumergic striatal interneurons. Nuclei were stained with DAPI (blue) as counterstain.

### Anti-D5 Dopamine Receptor (extracellular)

Product #: ADR-005

**Sizes:** 50 µl | 0.2 ml  
**Host:** Rabbit  
**Epitope:** EEGWELEGRTENC corresponding to amino acid residues 199-211 of rat D5 Dopamine receptor (Accession P25115).  
**Applications:** WB, IHC

**Immunohistochemical staining of D5 Dopamine Receptor in rat striatum**

- A. Perfusion-fixed brain frozen sections were stained with Anti-D5 Dopamine Receptor (extracellular) antibody (ADR-005) (1:100) (green fluorescence). D5 Dopamine receptor appears in a subset of striatal neurons and in the striatal matrix.  
- B. Staining of the same section with calbindin D28k (red fluorescence), a marker of interneurons.  
- C. Merging the two images demonstrates that D5 Dopamine receptor partially overlaps with the population of calbindin containing striatal interneurons. Blue is DAPI counterstain.

### Western blot analysis

#### Western blot analysis of Galanin receptor type 3 in rat brain membrane:
1. Anti-Galanin Receptor type 3 (extracellular) antibody (AGR-013) (1:200).  
2. Anti-Galanin Receptor type 3 (extracellular) antibody, preincubated with the control peptide antigen.

#### Western blot analysis of mouse brain membranes:
1. Anti-D5 Dopamine Receptor (extracellular) antibody (ADR-003), (1:200).  
2. Anti-D5 Dopamine Receptor (extracellular), preincubated with the control peptide antigen.

#### Western blot analysis of rat striatum membranes:
1. Anti-D3 Dopamine Receptor (extracellular) antibody (ADR-005), (1:200).  
2. Anti-D3 Dopamine Receptor (extracellular), preincubated with the control peptide antigen.
**Anti-H₁ Histamine Receptor**

**Product #: AHR-001**

Sizes: 50 µl | 0.2 ml  
Host: Rabbit.  
Epitope: Peptide ((C)H5R0YVSGLH1NRE, corresponding to amino acids 396-409 of rat H₁ Histamine Receptor (Accession P31390).  
Applications: WB, IH

**Expression of H₁ Histamine Receptor in mouse brain**

A. Staining of H₁ Histamine receptor in mouse ventromedial hypothalamus (VMH) with Anti-H₁ Histamine Receptor antibody (#AHR-001). H₁ Histamine receptor (green fluorescence) appears in the outline of the VMH nucleus (arrows). B. DAPI (blue) counterstain labels all cell nuclei including VMH.

Western blot analysis of mouse brain membranes:  
1. Anti-H₁ Histamine Receptor antibody (#AHR-001) (1:400).  
2. Anti-H₁ Histamine Receptor, preincubated with the control peptide antigen.

**Anti-Rat H₂ Histamine Receptor (extracellular)**

**Product #: AHR-002**

Sizes: 50 µl | 0.2 ml  
Host: Rabbit.  
Epitope: Peptide RNGTRGGNDTFKC, corresponding to amino acids 161-173 of rat H₂ Histamine Receptor (Accession P25102).  
Applications: WB, IH, IFC

**Expression of H₂ Histamine Receptor in rat cerebellum**

A. Rat brain sections (frozen) were stained with Anti-H₂ Histamine Receptor antibody (#AHR-002) (1:100) (green fluorescence). H₂ Histamine Receptor was particularly expressed in dendrites of Purkinje cells (arrows). B. Staining with mouse anti-parvalbumin (red fluorescence) detected Purkinje cells and interneurons in the molecular layer. C. Merge of the two images demonstrates that the staining was restricted to dendrites of Purkinje cells. Cell nuclei were labeled with DAPI (blue) as counterstain.

Western blot analysis of rat heart membranes (lanes 1 and 3) and rat basophilic leukemia (RBL) cell lysates (lanes 2 and 4):  
1. 2. Anti-H₁ Histamine Receptor antibody (AHR-001) (1:200).  
3. 4. Anti-H₁ Histamine Receptor, preincubated with the control peptide antigen.

**Anti-H₃ Histamine Receptor**

**Product #: AHR-003**

Sizes: 50 µl | 0.2 ml  
Host: Rabbit.  
Epitope: Peptide (C)RTRLRLDGGREAGPE, corresponding to amino acids 228-242 of rat H₃ Histamine Receptor (Accession Q9QVN8).  
Applications: WB, IH

**Expression of H₃ Histamine Receptor in rat cerebellum**

Western blot analysis of rat brain membranes (lanes 1 and 3) and mouse brain membranes (lanes 2 and 4):  
1. 2. Anti-H₃ Histamine Receptor antibody (AHR-003) (1:200).  
3. 4. Anti-H₃ Histamine Receptor, preincubated with the control peptide antigen.
Anti-Human H₄ Histamine Receptor (extracellular)  
**Product #: AHR-004**

Sizes: 50 µl | 0.2 ml  
Host: Rabbit.  
Epitope: Peptide HTLFEDWGFKEIC, corresponding to amino acids 75-87 of human H₄ Histamine Receptor (Accession Q9H3N8).  
Applications: WB, IH

Western blot analysis of human chronic myelogenous leukemia (K562) (lanes 1 and 3) and human promyelocytic leukemia (HL-60) (lanes 2 and 4) cell lysates:  
1, 2. Anti-Human H₄ Histamine Receptor (extracellular) antibody (AHR-004) (1:400).  
3, 4. Anti-Human H₄ Histamine Receptor (extracellular), preincubated with the control peptide antigen.

FACS analysis of H₄ Histamine Receptor expression in live intact Jurkat (acute T-cell leukemia) cell lines

Western blot analysis of rat (lanes 1 and 3) and mouse (lanes 2 and 4) brain membranes:  
1, 2. Anti-mGluR3 (extracellular) antibody (AGC-012) (1:600).  
3, 4. Anti-mGluR3, preincubated with the control peptide antigen.

Fresh brain stem sections were stained with Anti-mGluR3 (extracellular) antibody (AGC-012), followed by goat anti-rabbit Alexa Fluor 555 secondary antibody (red staining). Staining is present in neuronal cell bodies (white arrows) in the brainstem nuclei. Hoechst 33342 (blue) is used as counterstain.

Expression of mGluR2 in rat brain

A. mGluR2 (green fluorescence) is visualized in the corpus callosum (CC) and hippocampal stratum oriens (OR).  
B. Glial fibrillary acidic protein (GFAP) (red fluorescence), a marker of astrocytes.  
C. Merge of the two images demonstrates expression of mGluR2 in astrocytes.  
Blue is DAPI nuclear counterstain.

Perfusion-fixed frozen brain sections were stained with Anti-mGluR2 (extracellular) antibody (AGC-011).  
A. mGluR2 (green fluorescence) is visualized in the corpus callosum (CC) and hippocampal stratum oriens (OR).  
B. Glial fibrillary acidic protein (GFAP) (red fluorescence), a marker of astrocytes.  
C. Merge of the two images demonstrates expression of mGluR2 in astrocytes.  
Blue is DAPI nuclear counterstain.

Western blot analysis of rat cerebellum (lanes 1 and 3) and cortex (lanes 2 and 4) membranes:

1, 2. Anti-mGluR2 (extracellular) antibody (AGC-011) (1:400).  
3, 4. Anti-mGluR2, preincubated with the control peptide antigen.

Western blot analysis of rat cerebellum (lanes 1 and 3) and cortex (lanes 2 and 4) membranes:

1, 2. Anti-mGluR2 (extracellular) antibody (AGC-011) (1:400).  
3, 4. Anti-mGluR2, preincubated with the control peptide antigen.
Anti-Prokineticin Receptor 1 (extracellular)

Product #: APR-041

Sizes: 50 µl | 0.2 ml
Host: Rabbit.
Epitope: Peptide (C)ENTTNTDFTSARD corresponding to amino acid residues 10-24 of rat Prokineticin receptor 1 (Accession Q8R416).
Applications: WB, IH, IFC

Immunohistochemical staining of mouse olfactory bulb brain sections

A. Staining of mouse olfactory bulb sections with Anti-Prokineticin Receptor 1 (extracellular) antibody (APR-041), (1:100) (green fluorescence) reveals intensely stained astrocyte-like cells in the glomeruli (G) and lightly stained astrocyte-like cells in the adjacent layer (arrows).
B. Merge of images of PKR1 (green), parvalbumin (red, a neuronal marker) and DAPI (blue) nuclear counterstain.

Immunohistochemical staining of frozen sections of rat dorsal root ganglion (DRG)

Sections were stained with Anti-Prokineticin Receptor 1 (extracellular) antibody (APR-041), (1:100) followed by Alexa-555-conjugated goat anti-rabbit secondary antibody. PKR1 (red staining) is expressed in DRG neurons. Cell nuclei are visualized with Hoechst 33342 (blue staining).

Western blot analysis of mouse brain lysate:
1. Anti-Prokineticin Receptor 1 (extracellular) antibody (APR-041), (1:200).
2. Anti-Prokineticin Receptor 1 (extracellular) antibody, preincubated with the control peptide antigen.

Flow cytometry analysis of PAR-4 expression in human neutrophil-like differentiated promyelocytic leukemia HL-60 cells

HL-60 cells were induced to differentiate into a mature neutrophil-like phenotype by incubation with dimethyl sulfoxide (DMSO) (1:25%) for three days. The neutrophil phenotype was confirmed by staining the cells with Anti-CD11b-PE antibody. Double staining with Anti-PAR-4 (extracellular)-FITC antibody (APR-034-F), (1:40) shows that most differentiated cells express both markers (upper right panel).

Anti-Proteinase-Activated Receptor-4 (extracellular)-FITC

Product #: APR-034-F

Sizes: 50 µl
Host: Rabbit.
Epitope: Peptide (C)HLRGQRWPFGEAA(S)R corresponding to amino acid residues 136-150 of human PAR-4 (Accession Q96RI0). Cys 149 was replaced with Ser.
Applications: FC

Flow cytometry analysis of PAR-4 expression in live intact HL-60 (human promyelocytic leukemia) cell line

HL-60 cells + Anti-Proteinase-Activated Receptor-4 (extracellular)-FITC antibody (#APR-034-F).

Anti-α1B-Adrenoceptor-ATTO-488 (extracellular)

Product #: AAR-018-AG

Sizes: 50 µl
Host: Rabbit.
Epitope: Peptide (C)KNANFTGPNQTSSNS corresponding to amino acid residues 21-35 of human α1B-adrenoceptor (Accession P35368).
Applications: IC, FC

Flow cytometry analysis of α1B-Adrenoceptor in living GH3 cells

Unstained GH3 cells.
Cells + Anti-α1B-Adrenoceptor-ATTO-488 (extracellular) antibody (#AAR-018-AG), (1:100) (green). Nuclear staining of cells using the cell-permeable dye Hoechst 33342 (blue).

Extracellular staining of cells with Anti-α1B-Adrenoceptor-ATTO-488 (extracellular) antibody (#AAR-018-AG), (1:100) (green). Nuclear staining of cells using the cell-permeable dye Hoechst 33342 (blue).
**Anti-α_{1B}-Adrenoceptor (extracellular)**

**Product #: AAR-018**

Sizes: 50 µl | 0.2 ml  
Host: Rabbit.  
Epitope: Peptide (C)KNANFTGPNQTSSNS corresponding to amino acid residues 21-35 of human α_{1B}-adrenoceptor (Accession P35368).  
Applications: WB, IC, IFC

**Immunocytochemical staining of α_{1B}-Adrenoceptor in living GH3 cells**

Western blot analysis of α_{1B}-adrenoceptor in lysates of rat brain (lanes 1 and 3), rat kidney (lanes 2 and 4) and GH3 cell line (lanes 5 and 6).

1. Anti-α_{1B}-Adrenoceptor (extracellular) antibody (AAR-018), (1:200).
2. Anti-α_{1B}-Adrenoceptor (extracellular) antibody, preincubated with the control peptide antigen.

**Anti-Angiotensin-(1-7) Mas Receptor**

**Product #: AAR-013**

Sizes: 50 µl | 0.2 ml  
Host: Rabbit.  
Epitope: Peptide (C)KIRKNTWASHSSK corresponding to amino acid residues 212-224 of the rat Angiotensin-(1-7) Mas receptor (Accession P12526).  
Applications: WB, IH

**Expression of Angiotensin-(1-7) Mas Receptor in rat kidney**

Immunohistochemical staining of paraffin embedded sections of rat kidney using Anti-Angiotensin-(1-7) Mas Receptor antibody (AAR-013) (1:100).

Angiotensin-(1-7) Mas receptor is visualized (brown staining) in proximal tubules (PT) and distal tubules (DT) in the renal cortex. Note that collecting ducts (CD) are less stained and both glomeruli (G) and blood vessels (A) are negative. Counterstain is Hematoxilin.

**Anti-5-Hydroxytryptamine Receptor 1A**

**Product #: ASR-021**

Sizes: 50 µl | 0.2 ml  
Host: Rabbit.  
Epitope: Peptide KKSLNGQPGSGDWRRC corresponding to amino acid residues 251-266 of rat 5-Hydroxytryptamine Receptor 1A (Accession P19327).  
Applications: WB

**Western blot analysis of 5-Hydroxytryptamine Receptor 1A in lysates of mouse brain membrane (lanes 1 and 3) and rat brain membrane proteins (lanes 2 and 4):**

1. Anti-5-Hydroxytryptamine Receptor 1A antibody (ASR-021), (1:200).
2. Anti-5-Hydroxytryptamine Receptor 1A antibody, preincubated with the control peptide antigen.
Anti-Aquaporin 9

**Product #: AQP-009**

Sizes: 50 µl | 0.2 ml
Host: Rabbit.
Epitope: Peptide (C)EKDGAKKSMLQRLALK corresponding to residues 4-19 of rat AQP-9 (Accession number P56627).
Applications: WB, IH

**Expression of Aquaporin 9 in rat liver**

Immunohistochemical staining of paraffin embedded sections of rat liver using Anti-Aquaporin 9 antibody (#AQP-009) (1:100). Aquaporin 9 (brown staining) is expressed in hepatocytes of the liver parenchyma. Counterstain is Hematoxilin.

**Expression of Aquaporin 9 in rat epididymus**

Immunohistochemical staining of paraffin embedded section of rat testes using Anti-Aquaporin 9 antibody (#AQP-009) (1:100). Aquaporin 9 (brown staining) is expressed in the columnar epithelium of the epididymus (arrows). Counterstain is Hematoxilin.

**Western blot analysis of mouse brain membranes:**
2. Anti-Aquaporin 9, preincubated with the control peptide antigen.

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**BDNF - proDomain (WT-human)**  
**(pdBDNF-h)** recombinant, E. coli

**Product#: B-245**

Sizes: 10 µg | 5 x 10 µg

**NGF - proDomain (WT-human)**  
**(pdNGF-h)** recombinant, E. coli

**Product#: N-290**

Sizes: 10 µg | 5 x 10 µg

**Schematic description of BDNF precursor and the location of the prodomain region.**

**Schematic description of NGF precursor and the location of the prodomain region.**

---

**proBDNF (mut-human)** recombinant, E. coli

**Product#: B-256**

Sizes: 1 µg | 5 x 1 µg | 5 µg

**proBDNF (mut-human) activates MAPK in TrkB expressing cells**

TrkB was stably expressed in HEK-293 cells. Cells were then serum deprived for 2 hours followed by a 10 min treatment with or without the following; proBDNF (mut-human) (FB-256), proBDNF (WT-H) (FB-257) proBDNF (mut-m) (FB-243) or hBDNF (FB-250). Cell proteins were resolved by SDS-PAGE and the level of ERK1/2 phosphorylation was determined using anti-phosphoERK.

**proBDNF (WT-human)** recombinant, E. coli

**Product#: B-257**

Sizes: 1 µg | 5 x 1 µg | 5 µg

**proBDNF (WT-human) mediates neurite outgrowth in TrkB transfected PC12 cells**

PC12 cells were transiently transfected with TrkB/pcDNA3 containing the green fluorescent protein (GFP) as a reporter. One day post transfection, the cells were stimulated with 20ng/ml proBDNF (WT-human) (FB-257) or 10ng/ml hBDNF (FB-250). Development of neurites was visualized after 6 days using bright light microscopy.

---

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rCalciseptine
L-Type Ca²⁺ Channel Blocker

Product#: RTC-500

Sizes: 0.1 mg | 5 x 0.1 mg

rCalciseptine blocks L-type Ca²⁺ current in Xenopus oocytes

rCalciseptine (300 nM) inhibits L-type Ca²⁺ channels in Xenopus oocytes. The left panel shows the I-V relationship before and during application of 300 nM rCalciseptine (RTC-500). The right panel displays the traces of control and inhibited current.

---

rSNX-482
R-Type Ca²⁺ (Ca_{2.3}) Channel Blocker

Product#: RTS-500

Sizes: 5 µg | 5 x 5 µg | 10 µg | 5 x 10 µg

Recombinant SNX-482 inhibits Ca_{2.3} currents in Xenopus oocytes

Recombinant SNX-482 (100 nM, 200 nM, 400 nM) inhibits R-type Ca²⁺ currents in Xenopus oocytes. The left panel shows representative current traces before and following the application of 100, 200, and 400 nM rSNX-482 (RTS-500) (as indicated). The right panel displays the dose response for rSNX-482 (n=2-6).

---

Ca_{2.3} channel subunits α_{2.3}, β_{2.3}, and δ_{2.3} were co-expressed in Xenopus oocytes. Using TEVC, membrane potential was held at -100 mV. Ba²⁺ (10 mM) currents via Ca_{2.3} channels were elicited by 40 ms long voltage ramps from -100 mV to -60 mV, delivered every 10 seconds. Left: Representative current traces before and following the application of 100, 200, and 400 nM rSNX-482 (RTS-500) (as indicated). Right: Dose response for rSNX-482 (n=2-6).
**rPsalmotoxin 1 (rPcTx1)**

A novel and potent specific blocker of ASIC1a channel

Product#: RTP-100

Sizes: 5 µg | 5 x 5 µg | 10 µg | 5 x 10 µg | 0.1 mg

rPsalmotoxin inhibits the cation current of ASIC1a expressed in *Xenopus* oocytes

---

**Phrixotoxin-1**

*Kv4.2 and Kv4.3 Potent Blocker*

Product#: P-710

Sizes: 5 µg | 5 x 5 µg

Phrixotoxin-1 inhibits the K⁺ current of Kv4.2 channel expressed in *Xenopus* oocytes

---

**Phrixotoxin-3**

*Nah 1.2 Potent Blocker*

Product#: P-720

Sizes: 5 µg | 5 x 5 µg

Phrixotoxin-3 inhibits the Na⁺ current of Na₁.2 channel expressed in *Xenopus* oocytes

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**sApamin**

Small conductance Ca²⁺ Activated K⁺ Channel Potent Blocker

Product#: STA-200

Sizes: 0.5 mg | 5 x 0.5 mg | 1 mg | 5 x 1 mg

sApamin inhibits small conductance Ca²⁺ Activated K⁺ Channel (SK) current

---

**sApamin inhibits small conductance Ca²⁺ Activated K⁺ Channel (SK) current**

Voltage clamped currents of whole oocytes expressing rK₉.2.1 were recorded continuously at low Cl⁻ concentration at a 5 mV holding potential. At the indicated time, 10 nl of 100 mM Ca²⁺ was injected into the oocyte and an outward current developed. After one minute 1 µM sApamin (STA-200) was perfused to the bath, resulting in about 66% inhibition in the amplitude of this Ca²⁺-dependent current, which completely recovered upon toxin wash.

---

**Superimposed traces of hNa 1.5 currents measured in stably transfected HEK 293 cells using whole-cell voltage clamp technique under control (black) conditions and following 100 sec perfusion with 10nM, 25nM, 50nM and 100nM rProtoxin-1 (RTP-200) as indicated. Na 1.5 currents were elicited by 50 ms long voltage ramp from the resting potential of -100mV to +60 mV, delivered every 10 sec.**

---

**Superimposed traces of hNa 1.5 currents measured in stably transfected HEK 293 cells using whole-cell voltage clamp technique under control (black) conditions and following 100 sec perfusion with 10nM, 25nM, 50nM and 100nM rProtoxin-1 (RTP-200) as indicated. Na 1.5 currents were elicited by 50 ms long voltage ramp from the resting potential of -100mV to +60 mV, delivered every 10 sec.**

---

**Superimposed traces of Na 1.2 channel currents before (green) and during (black) application of 300 nM phrixotoxin-3 (P-720). Currents were elicited from a holding potential of -100 mV by voltage ramps of 35 ms to +60mV, delivered every 5 seconds.**

---

**Voltage clamped currents of whole oocytes expressing rK₉.2.1 were recorded continuously at low Cl⁻ concentration at a 5 mV holding potential. At the indicated time, 10 nl of 100 mM Ca²⁺ was injected into the oocyte and an outward current developed. After one minute 1 µM sApamin (STA-200) was perfused to the bath, resulting in about 66% inhibition in the amplitude of this Ca²⁺-dependent current, which completely recovered upon toxin wash.**
## General Ordering Information

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