

# Live or Let Die in Neuroscience: The Story of the Proneurotrophins

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Although it has been more than forty years since the neurotrophins were first discovered we are only now starting to appreciate the complexity of their true nature. These compounds lead a fascinating double life: as the proneurotrophin they bind to p75<sup>NTR</sup> and sortilin to set in motion diverse signaling pathways, which may lead to either apoptosis or cell survival, whereas in their incarnation as mature neurotrophins they bind to Trk and p75<sup>NTR</sup> receptors and insure cell survival.

The neurotrophins are a family of soluble, basic growth factors which regulate neuronal development, maintenance, survival and death in the CNS and the PNS.<sup>1</sup> They include nerve growth factor (NGF), the first member of the family to be discovered,<sup>2</sup> brain derived growth factor<sup>3</sup> (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4).<sup>4</sup> Their molecular weights are 13-15 kDa, having isoelectric points between 9 and 10. All of the neurotrophins form noncovalently linked homodimers in solution, with three intrachain disulfide bonds.<sup>5</sup> They bind to two types of receptors: each neurotrophin binds to a specific tyrosine kinase (Trk) receptor (NGF to TrkA, BDNF and NT4 to TrkB and NT3 to TrkC),<sup>6</sup> and all neurotrophins bind to the p75 neurotrophin receptor (p75<sup>NTR</sup>) receptor.<sup>7</sup> The residues involved in binding of neurotrophin to Trk receptors are different than those involved in binding to p75<sup>NTR</sup>.

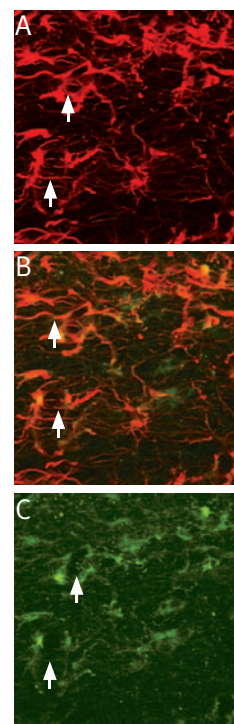
Binding of a neurotrophin to its specific Trk receptor activates its tyrosine kinase, leading to the activation of PI3K (phosphatidylinositol-3-kinase), MAPK (mitogen activated protein kinase), and phospholipase C- $\gamma$  pathways. Activation of these pathways leads to cell survival, differentiation, neurite growth, and activity dependent plasticity.<sup>8</sup> However, binding of neurotrophin to the p75<sup>NTR</sup> receptor leads to the activation of different pathways. p75<sup>NTR</sup> lacks an intrinsic catalytic activity, so its signaling is mediated by interaction with various cytoplasmic interactors. NADE (p75<sup>NTR</sup> NTR-associated cell death executor), NRIF (neurotrophin receptor-interacting factor) and NUAGE (neurotrophin receptor interacting MAGE homolog) are involved in the induction of cell death. NRIF, NUAGE and Schwann cell factor-1(SC-1) are involved in cell cycle arrest. The GTPase RhoA modulates neurite outgrowth. The activation of the NF- $\kappa$ B pathway

mediates cell survival.<sup>8</sup> Thus binding to p75<sup>NTR</sup> can either result in cell survival or cell death, depending on the particular cytoplasmic factor that has been recruited and the cellular context.<sup>9</sup> Ultimately the final decision about a cell's fate must take into account the ratio of Trk to p75<sup>NTR</sup> receptors.

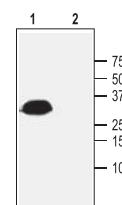
The neurotrophins are synthesized as precursor proteins, 240-260 amino acids long, which are further processed until they are secreted as mature homodimeric proteins into the extracellular space.<sup>10</sup> It was thought that the roles of the prodomain were to aid in the folding of the mature protein<sup>11</sup> and to sort the mature neurotrophin into the various secretion pathways.<sup>12</sup> The proneurotrophin is cleaved to the mature neurotrophin by various proteases: plasmin, furin, MP7, PC1/3, PC2, PC5/6, PC7, PACE4, which cut behind a pair or even a single basic residue.<sup>10</sup>

A paper by Lee *et al.* (2001) revealed a new role for the proneurotrophins.<sup>13</sup> They found that proNGF and not mature NGF is the ligand preferred by p75<sup>NTR</sup>. Thus they postulated p75<sup>NTR</sup>-induced cellular processes would be more efficiently induced by proNGF than by mature NGF. Indeed, they found that cleavage resistant proNGF (mutated at the cleavage site to insure that it retains the prodomain) induced apoptosis ten times more effectively than mature NGF in a vascular smooth muscle cell line expressing p75<sup>NTR</sup> but not Trk receptors. The ability of proNGF to induce apoptosis was confirmed by work showing that proNGF induces p75<sup>NTR</sup> mediated death of oligodendrocytes following spinal cord injury.<sup>14</sup> However, proNGF can also mediate survival. Rattenholl *et al.* (2001) have shown that proNGF was as effective as NGF in

## Expression of proNGF in Rat Glial Cells



ProNGF localization in glial cells of the rat corpus callosum. Staining of astrocytes was performed using mouse anti glial fibrillary acidic protein (GFAP), labeled red (A). Anti-proNGF antibody (#ANT-005) was labeled green (C). Co localization is demonstrated in B.



Western blotting with anti-proNGF antibody:

1. His-prodomain fusion protein, Anti-proNGF antibody (#ANT-005) (1:500).
2. His-prodomain fusion protein, Anti-proNGF antibody (1:500) preincubated with the control peptide antigen.

inducing survival of DRG neurons.<sup>11</sup> Fahnestock *et al.* (2004) have shown that a mutated proNGF exhibits neurite outgrowth on both PC12 cells and mouse sympathetic cervical ganglions.<sup>15</sup>

ProNGF is not the only proneurotrophin shown to have biological activity mediated by binding to p75<sup>NTR</sup>. Activation of p75<sup>NTR</sup> by proBDNF has been shown to facilitate hippocampal long term depression.<sup>16</sup> Most interestingly, a mutation in proBDNF has been shown to correlate with memory deficits in humans.<sup>17</sup>

The finding that proNGF, not mature NGF, is the predominant isoform associated with a variety of cell types, including mast cells, sciatic nerve cells, thyroid gland, skeletal muscle, prostate gland, hippocampus and hair follicle<sup>18</sup> underscores the biological significance of Lee *et al.*'s finding.

Expression of proBDNF has been found in many regions of the rat central nervous system including spinal cord, substantia nigra, amygdala, hypothalamus, cerebellum, hippocampus and cortex.<sup>19</sup> In the human cortex, pro-NGF appears to be the predominant isoform and is elevated in Alzheimer's disease.<sup>20</sup> In contrast, the level of proBDNF is decreased.<sup>21</sup>

Therefore, protease activity, controlling the balance between pro and mature neurotrophins becomes a major factor in the regulation of neurotrophin activity.

Several disease states, including stress and inflammation are known to affect the activity of proteases, and these also affect p75<sup>NTR</sup> expression and function.<sup>22</sup>

Thus, the neurotrophin story becomes even more complex. Not only the ratio of Trk receptors to p75<sup>NTR</sup> affects the cellular outcome, but also the ratio of mature to pro neurotrophin, controlled by protease activity.

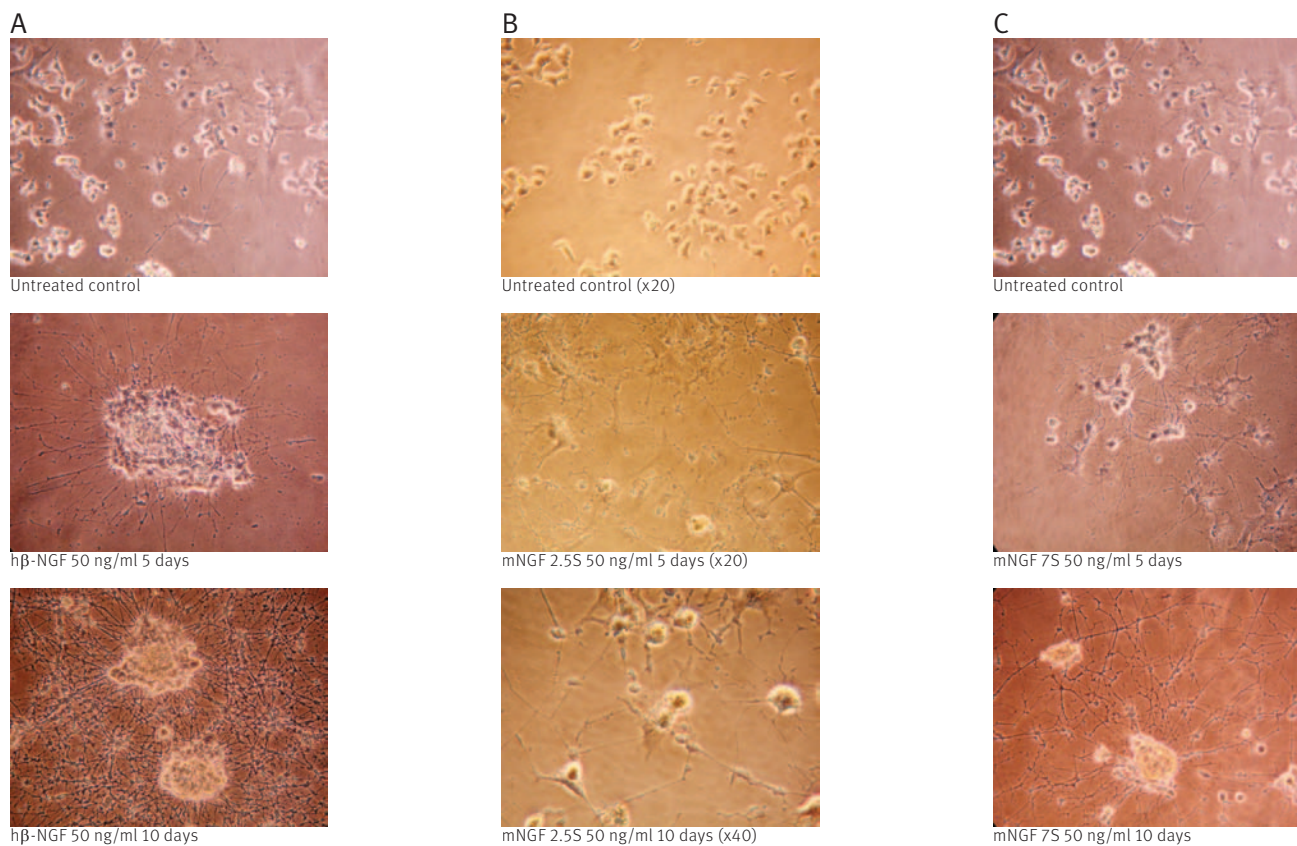
Not all cells which express significant levels of p75<sup>NTR</sup> undergo apoptosis in response to proNGF, suggesting that additional proteins are required. A solution to this dilemma was proposed in 2004 when sortilin was found to be a receptor for proneurotrophins but not mature neurotrophins.<sup>23</sup> Sortilin is a 95 kDa member of the Vps10p domain receptor family and is expressed in a variety of tissues, notably brain, spinal tissue and muscle. ProNGF binds to sortilin via its prodomain, and to p75<sup>NTR</sup> through residues in its mature domain. Thus a three membered complex between proNGF, sortilin and p75<sup>NTR</sup> is proposed as the signaling

intermediate required for proNGF mediated apoptosis. proBDNF also forms complexes with p75<sup>NTR</sup> and sortilin to elicit apoptosis in neurons.<sup>24</sup>

Although it has been more than forty years since the neurotrophins were first discovered, we are only now starting to appreciate the complexity of their true nature. These compounds lead a fascinating double life: as the proneurotrophin they bind to p75<sup>NTR</sup> and sortilin to set in motion diverse signaling pathways, which may lead to either apoptosis or cell survival, whereas in their incarnation as mature neurotrophins they bind to Trk and p75<sup>NTR</sup> receptors and insure cell survival.

Novel tools, such as antibodies to proneurotrophins, (**Anti-proNGF**, # ANT-005 **Anti-proBDNF**, #ANT-006) and antibodies to neurotrophin receptors **Anti-rat p75<sup>NTR</sup>** (#AN-170), **Anti-rat p75<sup>NTR</sup>-FITC** (#AN-170F), **Anti-Human p75<sup>NTR</sup>-extracellular** (#ANT-007), **Anti-Human p75<sup>NTR</sup>-extracellular-FITC** (#ANT-007-F), **Anti-Human p75<sup>NTR</sup>-intracellular** (#ANT-011) and **Anti-Sortilin** (ANT-009) will be essential for the numerous further studies which remain to be done in elucidating the control mechanisms which govern the balance between pro and mature neurotrophin, and ultimately the fate of the cell.

## NGF Mediated Neurite Outgrowth in Rat Pheochromocytoma Cells (PC12)

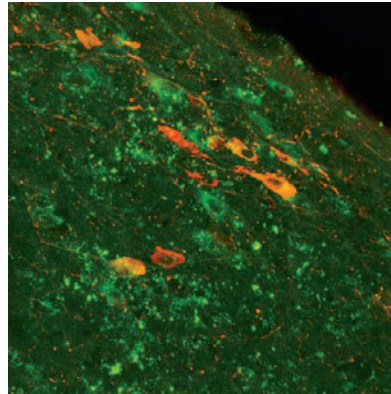


hβ-NGF (#N-235) (A); mNGF 2.5S (#N-240) (B); mNGF 7S (#N-130) (C); were bioassayed for neurotrophic activity using rat pheochromocytoma cell (PC12) cultures over a period of 10 days. PC12 cells were grown on collagen-coated 24 well plates in DMEM medium at 37°C and 7.5 % CO<sub>2</sub>. The cells were incubated for 24 hours before adding NGF solutions at the indicated concentration. The NGF solutions were changed every 2 or 3 days.

## References

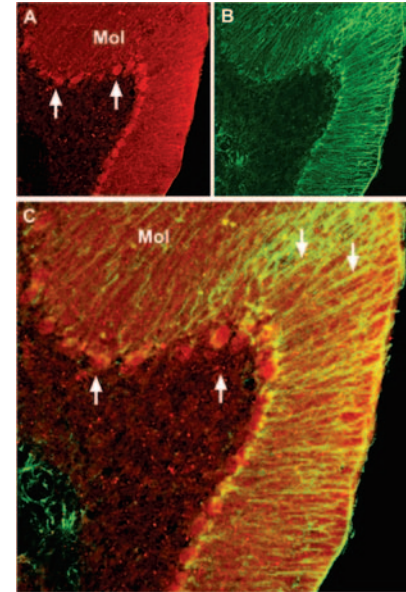
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## Anti-rat p75<sup>NTR</sup>-FITC



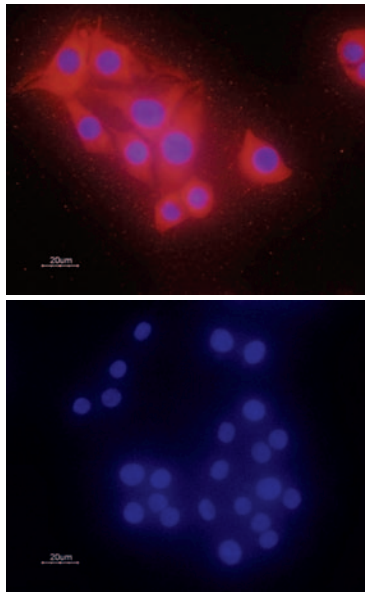
Immunohistochemical staining of the rat diagonal band nucleus, demonstrating partial co-localization of the p75<sup>NTR</sup> receptor using Anti-rat p75<sup>NTR</sup> antibody (#AN-170) and nitric oxide synthase (red fluorescence, Anti-NOS, Sigma).

## BDNF Expression in Mouse Cerebellum



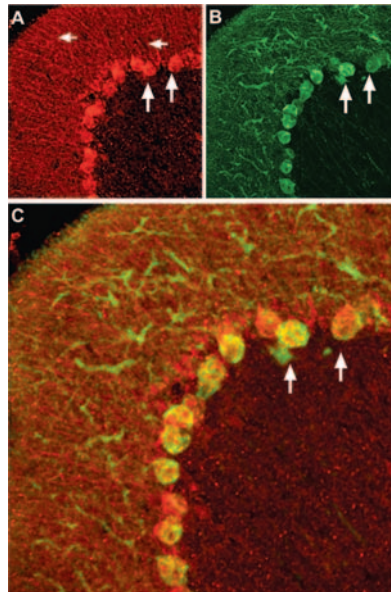
Immunohistochemical staining of brain derived neurotrophic factor (BDNF) with Anti-BDNF antibody (#ANT-010) in mouse cerebellum. A) BDNF (red) appears in Purkinje cells (upward pointing arrows) and is distributed diffusely in the molecular layer (Mol) including in astrocytic fibers (downward pointing arrows). B) staining of astrocytic fibers with glial fibrillary acidic protein (green) in the same section demonstrates the distribution of BDNF to neuronal as well as to astrocytic cellular components. C) Confocal merge of BDNF and GFAP.

## proBDNF Expression in C6 Rat Glioma



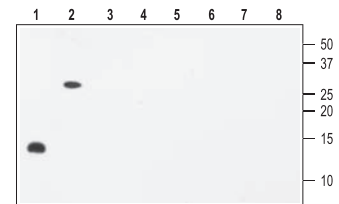
C6 glioma cells were fixed, permeabilized and stained with an Anti-proBDNF antibody (#ANT-006) followed by goat-anti-rabbit Alexa-555 secondary antibody (red staining) (upper panel). Specificity was confirmed by incubating the cells with the antibody preincubated with the peptide antigen (bottom panel). Cell nuclei were stained with the cell permeable dye Hoechst 33342 (blue staining).

## proBDNF Expression in Rat Cerebellum



Staining of pro-brain derived neurotrophic factor (proBDNF) with Anti-proBDNF antibody (#ANT-006) in rat cerebellum. Pro-BDNF (red) appears in Purkinje cell bodies (vertical arrows) and in astrocytic processes (horizontal arrows in A) but not in Purkinje neuronal dendrites stained for calbindin D28k (green, in B) in the same brain section. C, confocal merge of proBDNF and CBD28K demonstrates the restriction of proBDNF to Purkinje cell body.

## Specificity Testing of Anti-BDNF



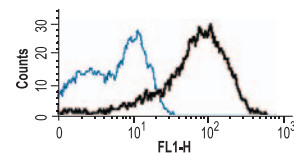
Western blotting with Anti-BDNF on:

- 1, 5. Recombinant human BDNF
  - 2, 6. Recombinant mouse proBDNF
  - 3, 7. Recombinant human NGF
  - 4, 8. Recombinant human NT-3
- Lanes 1-4 Anti-BDNF (#ANT-010) (1:200). Lanes 5-8 Anti-BDNF, preincubated with the control peptide antigen.

**Note:** The antibody recognizes both BDNF and proBDNF but fails to recognize the closely related NGF and NT-3 neurotrophins.

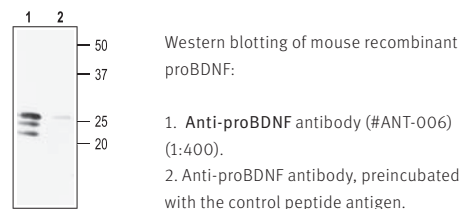
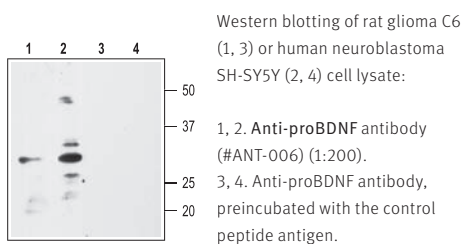
## Cell Surface Expression of p75<sup>NTR</sup>

### on PC12 Cells

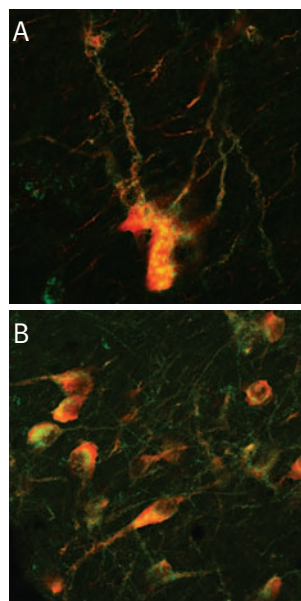


FACS analysis of PC12 cells:

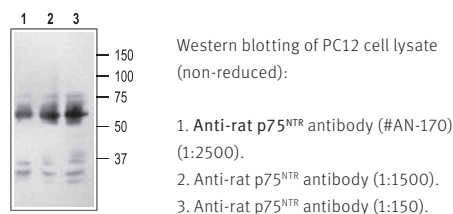
- Unstained cells
- Anti-rat p75-FITC (#AN-170-F) antibody



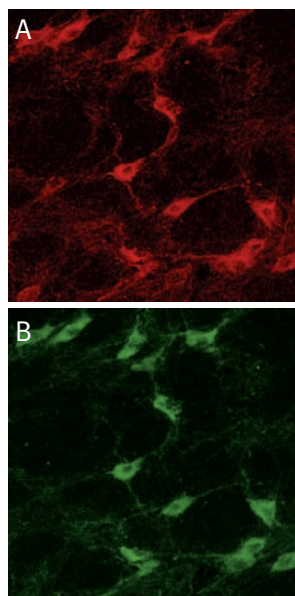
## Expression of p75<sup>NTR</sup> in Rat Brain



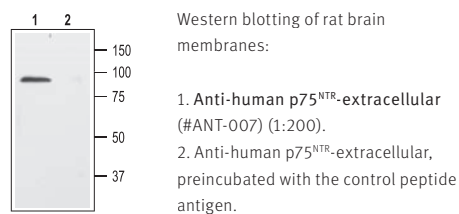
A: Distribution of the neurotrophin receptor p75 using Anti-rat p75<sup>NTR</sup> antibody (#AN-170) in the diagonal band of the rat basal forebrain shown by indirect immunofluorescence labelling. B: Exclusive co-expression of p75<sup>NTR</sup> (yellowish dots) in cholinergic projection neurons revealed by concomitant carbocyanine staining of choline acetyltransferase (ChAT, green). Picture obtained by a Zeiss LSM 510 Meta, from Wolfgang Hartig & Jens Grosche, PFI, (Leipzig Univ., Germany).



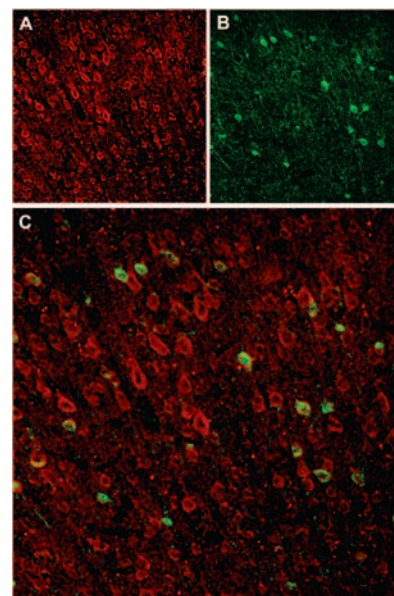
## Expression of p75<sup>NTR</sup> in Rat Brain



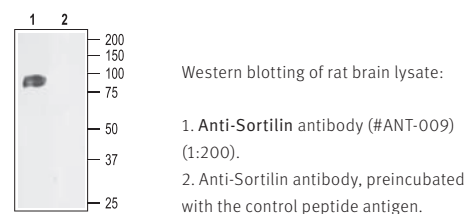
Staining of p75<sup>NTR</sup> with Anti-human p75<sup>NTR</sup>-extracellular (#ANT-007) in rat brain. In A, cells in the nucleus basalis magnocellularis are stained positive for p75<sup>NTR</sup>. In B, staining of the same section shown in A, with goat anti choline acetyltransferase (ChAT) confirms that p75<sup>NTR</sup> staining was specific to cholinergic neurons.



## Sortilin Expression in Rat Cortex



Immunohistochemical staining of Sortilin receptor with Anti-Sortilin antibody (#ANT-009) in rat neocortex. A, Sortilin receptor appears in cortical neurons (red). B, Staining of interneurons with mouse anti parvalbumin (PV, green). C, Confocal merge of Sortilin receptor and PV demonstrates separate localization in neocortex.



## Related Products

Compound	Product #
<b>Nerve Growth Factors</b>	
mNGF 2.5S (Grade I)	N-240
mNGF 2.5S (Grade II)	N-100
mNGF 7S	N-130
hβ-NGF	N-235
<b>Neurotrophins</b>	
hBDNF	B-250
hCardiotrophin-1	C-200
hGDNF	G-240
hNeurotrophin-3 (NT-3)	N-260
hNeurotrophin-4/5 (NT-4/5)	N-270
hPersephin	P-320

<b>Neurotrophic Cytokines</b>	
rCNTF	C-245
hLIF	L-200
mInterleukin-6	I-220
<b>Heparin-Binding Growth Factors</b>	
hFGF basic	F-170
hMidkine	M-135
hPleiotrophin	P-150
<b>Neurotrophic Factors EconoKit</b>	
A collection of all Neurotrophic Factors	EK-500

<b>Neurotrophin Antibodies</b>	
Anti-BDNF	ANT-010
Anti-proBDNF	ANT-006
Anti-mNGF	AN-240
Anti-NT-3	ANT-003
Anti-NT-4	ANT-003
Anti-proNGF	ANT-005
Anti-Human p75 <sup>NTR</sup> -extracellular	ANT-007
Anti-Human p75 <sup>NTR</sup> -extracellular-FITC	ANT-007-F
Anti-Rat p75 <sup>NTR</sup>	AN-170
Anti-Rat p75 <sup>NTR</sup> -FITC	AN-170-F
Anti-Human p75 <sup>NTR</sup> -intracellular	ANT-011
Anti-Sortilin	ANT-009