

## ANTI-NEUROTROPHIN ANTIBODY SAMPLE PACK 2

**CATALOG NUMBER:** NS114

**LOT NUMBER:**

**QUANTITY:** 20 µL each of AB1528, AB1513, AB1517, AB1519 and Negative Control

**DESCRIPTION:** The fate of many neurons in the developing and adult nervous system is controlled partly by neurotrophins. This family of proteins include Nerve Growth Factor (NGF), Brain Derived Neurotrophic Factor (BDNF), Neurotrophin 3 (NT3) and Neurotrophin 4/5 (NT4/5).

Neurotrophins are known to regulate the survival and differentiation of selective populations of neurons during embryonic development and maintenance of specific function in adulthood. The secretion of neurotrophins supports the selection of neurons during the thinning of neuronal population by programmed cell death (apoptosis). However, the roles of these neurotrophic factors in the brain and peripheral tissues are not very well understood. Therefore the availability of antibodies capable of neutralizing each neurotrophin will greatly aid further exploration of the action of these factors.

CHEMICON's Anti-Neurotrophin Antibody Sample Pack 2 consists of whole serum sheep polyclonal antibodies for each of the four neurotrophins and a negative control and is conveniently packaged for use in biological inhibition analysis.

The Antibody Sample Pack 2 allows neutralization of neurotrophin activities for each of the neurotrophins. The levels of cross reactivity of each antibody for other members of the neurotrophin family is very low (Table 1).

**Table 1.** Neurotrophin antibody cross reactivities.

Antibody	ED <sub>50</sub> (µg/mL)				
	NGF	BDNF	NT3	NT4	Ig
NGF	+	-	-	+/-	-
	(0.6)				
BDNF	-	+	-	-	-
		(10.5)			
NT3	-	-	+	-	-
			(5.5)		
NT4	-	-	-	+	-
				(3.6)	
Ig	-	-	-	-	-

Note: (-) represents no cross reactivity between antibody and neurotrophin (ED<sub>50</sub> > 200 µg/mL).  
 (+) represents antibody neutralization of neurotrophin.  
 (+/-) represents slight cross reactivity.

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### Sample protocol for using Chemicon's neurotrophin antibodies to inhibit biological activities

#### Preparation of single neurons

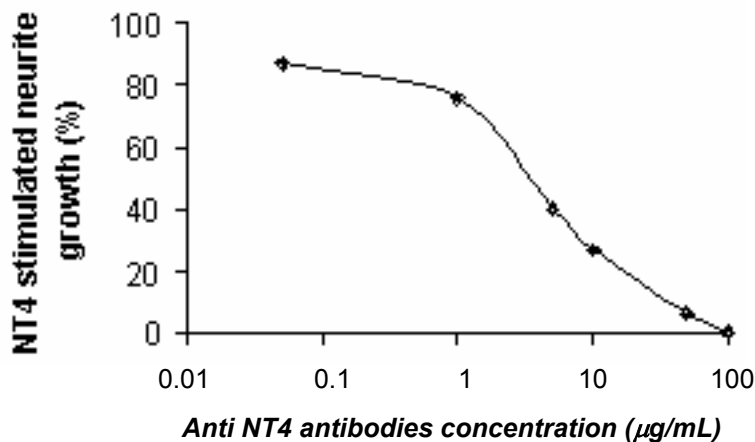
1. Dissect neural tissue to be studied (eg. dorsal root ganglia from E10 chick embryo) and place into calcium/magnesium free phosphate buffered isotonic saline (CMF-PBS).
2. Remove CMF-PBS.
3. Add fresh CMF-PBS containing 1% trypsin.
4. Incubate the tube at 37°C for 15-20 minutes and agitate at regular intervals.
5. Remove most of the liquid.
6. Wash tissue twice in Hanks containing 10% heat inactivated horse serum to inactivate residual trypsin.
7. Wash in CMF-PBS.
8. Remove liquid by pelleting tissue between washings via centrifugation at 2000xg for 1-2 min.
9. Dissociate tissue (trituration) by taking up the tissue in CMF-PBS with a siliconized pasture pipette heated to reduce tip diameter to a fine bore. Expel the contents while pressing the tip against the bottom of the tube.
10. Monitor trituration by examining a drop of the dissociated cells on a slide using an inverted phase contrast microscope.

#### Inhibition of neurotrophic activity with Chemicon's neutralizing antibodies

11. Preincubate for one hour at room temperature the neurotrophin at 10 ng/mL in BME with 1% fetal calf serum or 2 mg/mL of bovine serum albumin and anti-neurotrophin antibodies in increasing concentration (1-100 µg/mL is recommended).

Results can be plotted as shown below

*The effect of anti-NT4 antibody on the neurite outgrowth of embryonic DRG promoted by NT4.*



The ED50 of anti-NT4 neutralization is approximately 3.6 µg/mL

#### References:

Davis, A.M. (1989) Chapter 5: Neurotrophic factor bioassay using dissociated neurons. In Nerve Growth Factors, Edited by Rush, R.A. John Wiley and sons, New York.

Ebendal, T. (1989) Chapter 4: Use of collagen gels to bioassay nerve growth factor activity. In Nerve Growth Factors, Edited by Rush, R.A. John Wiley and sons, New York.

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**SHEEP ANTI-NERVE GROWTH FACTOR-BETA  
POLYCLONAL ANTIBODY**

- CATALOG NUMBER:** AB1528
- LOT NUMBER:**
- QUANTITY:** 20  $\mu$ L
- SPECIFICITY:** Nerve growth factor-beta (NGF). Less than 1% cross-reactivity against recombinant human NT4 and BDNF and approximately 2% for NT3 by ELISA.
- IMMUNOGEN:** Mouse nerve growth factor-beta purified from salivary glands.
- APPLICATIONS:** Immunohistochemistry at 1:500-1:5,000 (protocol available).  
Immunoblotting at 1:500-1:5,000.  
ELISA at 1:500-1:5,000.  
Inhibition of biological activity *in vitro* at 1:10-1:50.  
Use neat for *in vivo* animal studies (5-10  $\mu$ L/g body weight).  
Optimal working dilutions must be determined by end user.
- BIOLOGICAL ACTIVITY:** The antiserum will completely inhibit the survival and neurite outgrowth actions of mouse NGF in chicken dorsal root ganglion neurons *in vitro*.
- SPECIES REACTIVITIES:** Mouse, rat, chicken, and human NGF-beta. Does not react with bovine NGF.
- FORMAT:** Whole sheep antisera.
- PRESENTATION:** Lyophilized. Reconstitute with 20  $\mu$ L of sterile distilled water. Centrifuge to remove any insoluble material.
- STORAGE/HANDLING:** Maintain lyophilized material at -20°C for up to 12 months after date of receipt. After reconstitution maintain at -20°C to -70°C in undiluted aliquots for up to 6 months. Avoid repeated freeze/thaw cycles.
- REFERENCES:** *J. Neurosci. Meth.* (1994) **54**:95-102.  
*J. Neurochem.* (1996) **66**:2995-2999.

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**SHEEP ANTI-BRAIN DERIVED NEUROTROPHIC FACTOR  
POLYCLONAL ANTIBODY**

- CATALOG NO:** AB1513
- LOT NUMBER:**
- QUANTITY:** 20 µL
- SPECIFICITY:** Brain Derived Neurotrophic Factor (BDNF). By one site ELISA, less than 1% cross-reactivity against mouse NGF, recombinant human NT3 or neurotrophin 4.
- IMMUNOGEN:** Recombinant Human Brain Derived Neurotrophic Factor (BDNF).
- APPLICATIONS:** Immunohistochemistry: 1:200-1:2,000 (protocol available).  
Immunoblotting: 1:200-1:2,000  
ELISA: 1:200-1:2,000  
Inhibition of biological activity *in vitro*: 1:10-1:50.  
Use neat for *in vivo* animal studies (5-10 µL/g body weight).  
Optimal working dilutions must be determined by end user.
- BIOLOGICAL ACTIVITY:** Neutralizes BDNF, but not other neurotrophins.
- SPECIES REACTIVITIES:** Rat and human.
- FORMAT:** Sheep serum
- PRESENTATION:** Lyophilized, no preservatives. Reconstitute with 20 µL of sterile distilled water. Centrifuge to remove any insoluble material.
- STORAGE/HANDLING:** Maintain lyophilized material at -20°C for up to 12 months after date of receipt. After reconstitution maintain at -20°C to -70°C in undiluted aliquots for up to 6 months. Avoid repeated freeze/thaw cycles.

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**SHEEP ANTI-NEUROTROPHIN 3  
POLYCLONAL ANTIBODY**

- CATALOG NO:** AB1517
- LOT NUMBER:**
- QUANTITY:** 20 µL
- SPECIFICITY:** Neurotrophin 3 (NT3). By one site ELISA, less than 1% cross-reactivity against mouse NGF and recombinant human BDNF. Approximately 5% cross-reactivity against neurotrophin 4.
- IMMUNOGEN:** Recombinant Human Neurotrophin 3 (NT3).
- APPLICATIONS:** Immunohistochemistry: 1:200-1:2,000 (protocol available).  
Immunoblotting: 1:200-1:2,000  
ELISA: 1:200-1:2,000  
Inhibition of biological activity *in vitro*: 1:10-1:50  
Use neat for *in vivo* animal studies (5-10 µL/g body weight).  
Optimal working dilutions must be determined by the end user.
- BIOLOGICAL ACTIVITY:** The antiserum will neutralize NT3, but not other neurotrophins.
- SPECIES REACTIVITIES:** Rat, chicken and human.
- FORMAT:** Sheep serum
- PRESENTATION:** Lyophilized, no preservatives. Reconstitute with 20 µL of sterile distilled water. Centrifuge to remove any insoluble material.
- STORAGE/HANDLING:** Maintain lyophilized material at -20°C for up to 12 months after date of receipt. After reconstitution maintain at -20°C to -70°C in undiluted aliquots for up to 6 months. Avoid repeated freeze/thaw cycles.

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**SHEEP ANTI-NEUROTROPHIN 4  
POLYCLONAL ANTIBODY**

- CATALOG NO:** AB1519
- LOT NUMBER:**
- QUANTITY:** 20 µL
- SPECIFICITY:** Neurotrophin 4 (NT4). By one site ELISA, less than 1% cross-reactivity against mouse NGF and recombinant human BDNF. Approximately 5% cross-reactivity against neurotrophin 3.
- IMMUNOGEN:** Recombinant Human Neurotrophin 4 (NT4).
- APPLICATIONS:** Immunohistochemistry: 1:500-1:2,000 (protocol available).  
Immunoblotting: 1:500-1:2,000  
ELISA: 1:500-1:2,000  
Inhibition of biological activity *in vitro*: 1:10-1:50  
Use neat for *in vivo* animal studies (5-10 µL/g body weight).  
Optimal working dilutions must be determined by the end user.
- BIOLOGICAL ACTIVITY:** The antiserum will neutralize NT4, but not other neurotrophins.
- SPECIES REACTIVITIES:** Human, rat, mouse and monkey. Reactivity with other species has not been determined.
- FORMAT:** Sheep serum.
- PRESENTATION:** Lyophilized, no preservatives. Reconstitute with 20 µL of sterile distilled water. Centrifuge to remove any insoluble material.
- STORAGE/HANDLING:** Maintain lyophilized material at -20°C for up to 12 months after date of receipt. After reconstitution maintain at -20°C to -70°C in undiluted aliquots for up to 6 months. Avoid repeated freeze/thaw cycles.

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## NEGATIVE CONTROL

<b>CATALOG NO:</b>	NS114-nc
<b>LOT NUMBER:</b>	xxxxxxx
<b>QUANTITY:</b>	20 µL
<b>SPECIFICITY:</b>	Sheep serum from an unimmunized animal.
<b>APPLICATIONS:</b>	For use as a negative control for the NS114 kit. Optimal working dilutions must be determined by the end user.
<b>SPECIES REACTIVITIES:</b>	No reactivity with rat. Other species have not yet been tested.
<b>FORMAT:</b>	Sheep serum.
<b>PRESENTATION:</b>	Lyophilized, no preservatives. Reconstitute with 20 µL of sterile distilled water.
<b>STORAGE/HANDLING:</b>	Maintain lyophilized material at -20°C for up to 12 months after date of receipt. After reconstitution maintain at -20°C to -70°C in undiluted aliquots for up to 6 months. Avoid repeated freeze/thaw cycles. Glycerol (ACS grade or better) can be added (1:1) for additional stability.

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