

ANTI-NEUROTROPHIN ANTIBODY SET 2

CATALOG NUMBER: NS116

LOT NUMBER: xxxxxxxxx

QUANTITY: 100 μ L 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 Set 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 Set 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 ₅₀ (μ g/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 (ED₅₀ > 200 μ g/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

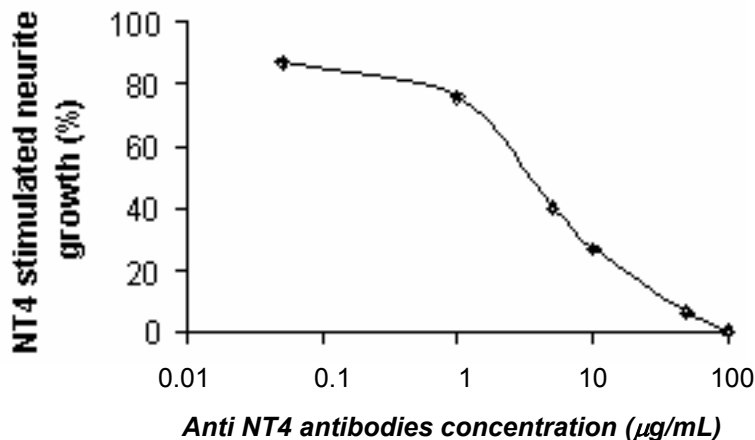
1. Dissect neural tissue to be studied (eg. dorsal root ganglia from E10 chick embryo) and place into calcium/magnesium free phosphate buffered isotonic saline (CMF-PBS).
2. Remove CMF-PBS.
3. Add fresh CMF-PBS containing 1% trypsin.
4. Incubate the tube at 37°C for 15-20 minutes and agitate at regular 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.

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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:** xxxxxxxx
- QUANTITY:** 100 µL
- 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*: 1:10-1:50
Inhibition of biological activity *in vivo*: 5-10 µL/gram 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 100 µL of sterile distilled water.
- STORAGE/HANDLING:** Maintain lyophilized material at -20 to -70°C for up to one year from date of receipt. After reconstitution maintain at -20 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.
- 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:** xxxxxxxx
- QUANTITY:** 100 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 100 µL of sterile distilled water.
- STORAGE/HANDLING:** Maintain lyophilized material at -20 to -70°C for up to one year from date of receipt. After reconstitution maintain at -20 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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**SHEEP ANTI-NEUROTROPHIN 3
POLYCLONAL ANTIBODY**

- CATALOG NO:** AB1517
- LOT NUMBER:** xxxxxxxx
- QUANTITY:** 100 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, human and chicken.
- FORMAT:** Sheep serum
- PRESENTATION:** Lyophilized, no preservatives. Reconstitute with 100 µL of sterile distilled water.
- STORAGE/HANDLING:** Maintain lyophilized material at -20 to -70°C for up to one year from date of receipt. After reconstitution maintain at -20 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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**SHEEP ANTI-NEUROTROPHIN 4
POLYCLONAL ANTIBODY**

- CATALOG NO:** AB1519
- LOT NUMBER:** xxxxxxxx
- QUANTITY:** 100 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: 1:200-1:2,000 (protocol available).
Immunoblotting: 1:200-1:2,000
ELISA: 1:200-1:2,000
Inhibition of biological activity *in vitro*: 1:10-1:50
Inhibition of biological activity *in vivo*: 5-10 µL/gram 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.
- FORMAT:** Sheep serum.
- PRESENTATION:** Lyophilized, no preservatives. Reconstitute with 100 µL of sterile distilled water.
- 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. Glycerol (ACS grade or better) can be added (1:1) for additional stability.
- REFERENCE:** Zhang et al., *J. Neurosci. Meths.* (1999) **89**(1):69.
- GENERAL REFERENCE:** Lodouichi, *J. Neurosci.* (2000) **20**(6):2155.

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NEGATIVE CONTROL

CATALOG NO:	NS116-nc
LOT NUMBER:	xxxxxxxx
QUANTITY:	100 μ L
SPECIFICITY:	Sheep serum from an unimmunized animal.
APPLICATIONS:	For use as a negative control for the NS116 kit. Optimal working dilutions must be determined by the end user.
SPECIES REACTIVITIES:	No reactivity with rat. Other species have not yet been tested.
FORMAT:	Sheep serum.
PRESENTATION:	Lyophilized, no preservatives. Reconstitute with 100 μ L of sterile distilled water.
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. Glycerol (ACS grade or better) can be added (1:1) for additional stability.

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