

Product Contents

Nerve Growth Factor, 2.5S, Murine:

Part No.	Size
G514A	100µg

Description: Murine 2.5S Nerve Growth Factor (2.5S mNGF) is a 26kDa protein composed of two identical 118 amino acid chains. This factor mediates phosphorylation of specific intracellular proteins (1). Target cells of this molecule include sympathetic and sensory neurons and derivatives of nerve cells such as adrenal medulla pheochromocytoma (PC12) cells. Murine 2.5S Nerve Growth Factor is expressed in sympathetic and sensory-innervated peripheral tissues such as the vas deferens, heart, iris, skin, splenic capsule, sciatic nerve and submaxillary gland (2,3). At the cellular level, mNGF expression has been demonstrated in lymphocytes (4), smooth muscle cells, epithelial cells, astrocytes, fibroblasts and Schwann cells (5). There is a strong correlation between the levels of NGF mRNA in peripheral tissues and the density of sympathetic innervation (5). Peripheral neurons that respond to mNGF include adrenergic sympathetic neurons and primary sensory neurons (6). Cellular responses to this neurotrophic factor include differentiation, neuritic extension and production of catecholamine and neuropeptide-synthesizing enzymes (7). The 2.5S form originates from the dissociation and proteolytic cleavage of the β subunit of 7S NGF (8) and is also referred to as β -NGF. This form is solely responsible for the biological activity of both the 2.5S and 7S forms of this neurotrophic factor (9). Murine Nerve Growth Factor binds to the "low-affinity" NGF receptor (p75^{NTR}; 10), as do all of the neurotrophic factors, and it mediates, via p75^{NTR} signal transduction, the functional responses of Schwann cells (11). mNGF also activates signal transduction by the dimerization and autophosphorylation of the TrkA receptor (also known as Trk and p140^{Trk}; 12). For a review on TrkA signaling, see references 13 and 14. Murine 2.5S Nerve Growth Factor is purified from male mouse submaxillary glands by the method of Bocchini and Angeletti (15).

Formulation: Murine 2.5S Nerve Growth Factor is supplied as a sterile-filtered, lyophilized powder.

Isoelectric Point: pI 8.0.

Solubility: The lyophilized 2.5S mNGF has a solubility of approximately >1mg/ml in neutral or acidic solutions.

Storage Conditions: Store desiccated at -20°C. See the expiration date on the product information label. Store reconstituted 2.5S mNGF in aliquots at -20°C, where it is stable for six months. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.

Quality Control Assays

Biological Activity: The ED₅₀ for Murine 2.5S Nerve Growth Factor, i.e., the concentration of factor that produces one-half the maximal response, is determined using the serum-free medium bioassay for PC12 cells (16) and the CellTiter 96[®] AQ_{UBOUS} Non-Radioactive Cell Proliferation Assay (Cat.# G5421). The ED₅₀ value obtained is reported on the Product Information Label attached to this document.

Specific Activity: Specific activity of Murine 2.5S Growth Factor is assigned by direct comparison with the reference standard (#93/556) from the National Institute for Biological Standards and Controls (NIBSC)^(a). The specific activity obtained with this lot is reported on the Product Information Label attached to this document.

Usage Information

I. Bioactivity Determination of Murine NGF Using the PC-12 (Pheochromocytoma) Cell Line

The following protocol is used by Promega to test the activity of 2.5S mNGF preparations. With modifications, this protocol can be used for cell proliferation assays in a variety of experimental applications. Alternatively, NGF activity can be measured using choline acetyltransferase (ChAT) activity in rat basal forebrain primary septal cell cultures as described in reference 17.

Materials to Be Supplied by the User

(Solution compositions are provided in Section II.)

- PC-12 cells (ATCC# CRL 1721)
- CellTiter 96[®] AQ_{UBOUS} Non-Radioactive Cell Proliferation Assay (Cat.# G5421)
- complete medium
- trypsin/EDTA
- Mg²⁺- and Ca²⁺-free Dulbecco's PBS (DPBS)
- rat tail collagen type I in DPBS
- NGF diluent
- 96-well plates

Part# 9PIG514

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^(a)Address for NIBSC is Blanche Lane, South Mimms Potters Bar, Hertfordshire EN6 3QG, United Kingdom.

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A. Protocol

This protocol uses the CellTiter 96[®] AQ_{U96US} Non-Radioactive Cell Proliferation Assay to determine bioactivity of 2.5S mNGF. A more detailed protocol for the CellTiter 96[®] AQ_{U96US} Assay is available, upon request, in Technical Bulletin #TB169.

- Maintain stock cultures of PC-12 cells with complete medium in Corning 75cm² flasks that have been coated with 6µg/cm² of rat tail collagen. Pass cells when they reach confluency by rinsing the flask with DPBS followed by trituration with complete medium. Reseed flasks at 2 × 10⁶ cells per flask.
- Coat a 96-well flat bottom plate with 50µl/well of collagen type I in DPBS (50µg/ml). Incubate the plate at 37°C for a minimum of 30 minutes.

Note: Plates can be prepared several days in advance. After coating the wells, remove the collagen, add 50–100µl/well of DPBS and store the plate in a 37°C cell culture incubator.

- Add 5ml of trypsin/EDTA to a confluent 75cm² flask of PC-12 cells and incubate for 2–3 minutes. Inactivate the trypsin by adding 5ml of complete medium to the cell suspension.
- Pellet the cells by centrifugation (5 minutes at 250 × g) and wash once with 10ml of serum-free RPMI medium (37°C).
- Resuspend the cells in 10ml of serum-free RPMI medium (37°C), count them and then suspend at a final density of 4 × 10⁵ cells/ml.
- Remove the collagen-coated 96-well plate from the incubator and aspirate the collagen solution using a multichannel pipette. **Hint:** Tilt the plate while removing the solution.
- Seed 36 wells with 3.6 × 10⁴ cells/well (90µl/well). Incubate cells at 37°C in a humidified 5% CO₂ atmosphere while preparing the 2.5S mNGF samples.
- Prepare the NGF solution in sterile deionized water (100ng/µl). In a separate 96-well plate, prepare a serial dilution (12 different concentrations in NGF diluent) of the 2.5S mNGF at 10X the final concentration to be used in the wells. The final assay concentration should range from 0 to 100ng/ml. Prepare at least 50µl of each concentration so that the assay can be performed in triplicate.
- Add 10µl of each 2.5S mNGF dilution to the test wells (in triplicate) containing the plated cells (final volume = 100µl/well).
- Incubate the plate for 20 hours at 37°C in a humidified 5% CO₂ atmosphere.
- Add 20µl/well of freshly prepared combined MTS/PMS solution.
- Incubate the plate for 5 hours at 37°C in a humidified 5% CO₂ atmosphere.

Note: To perform the colorimetric assay, proceed immediately to Step 13. Alternatively, to measure the absorbance at a later time, add 25µl of 10% SDS to each well to stop the reaction. Store the SDS-treated plates, protected from light, in a humidified chamber at room temperature for ≤18 hours.

- Record the absorbance at 490nm using an ELISA plate reader.
- Plot the absorbance at 490nm (Y axis) versus concentration of growth factor (X axis) and determine the ED₅₀ value.

II. Composition of Buffers and Solutions

trypsin/EDTA, 1X

0.05% (w/v)	trypsin
0.5mM	EDTA

Dissolve these components in a calcium- and magnesium-free salt solution such as 1X PBS or 1X HBSS Filter-sterilize (0.22µm pore).

complete medium

RPMI 1640 medium supplement to contain a final concentration of:

15mM	HEPES
10%	horse serum
5%	fetal bovine serum

DPBS (one liter)

0.2g/L	KCl
8.0g/L	NaCl
0.2g/L	KH ₂ PO ₄
1.15g	Na ₂ HPO ₄

NGF diluent (prepare fresh)

RPMI 1640 medium supplement to contain a final concentration of:

100µg/ml	BSA
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Filter-sterilize (0.22µm pore) and use immediately.

III. Related Products

Product	Size	Cat.#
NGF E _{max} [®] ImmunoAssay System	2 × 96 wells	G7630
	5 × 96 wells	G7631
Anti-NGF mAb	100µg	G1131
	20µg	G1132
CellTiter 96 [®] AQ _{U96US} Non-Radioactive Cell Proliferation Assay*	1,000 assays	G5421
	5,000 assays	G5430
	50,000 assays	G5440

*For Laboratory Use.

IV. References

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