Certificate of Analysis

Nerve Growth Factor, 2.5S, Murine:

Part No.	Size
G514A	100µg

Part# 9PIG514

Revised 1/19

Description: Murine 2.5S Nerve Growth Factor (2.5S mNGF) is a 26kDa protein composed of two identical 118 amino acid chains. Murine 2.5S Nerve Growth Factor is purified from male mouse submaxillary glands by the method of Bocchini and Angeletti (1).

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

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.

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Quality Control Assays

Biological Activity: The EC_{50} 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 (2).

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.

This lot passes the following Quality Control specifications:

Test Parameters	Specification	Result
EC_{50}	<2ng/ml	Passed

Promega

Promega Corporation	
2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
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Signed by:

R. Wheeler, Quality Assurance

[a]Address for NIBSC is Blanche Lane, South Mimms Potters Bar, Hertfordshire EN6 3QG, United Kingdom.

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Usage Information

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

The following protocol can be modified 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 3.

Materials to Be Supplied by the User
(Solution compositions are provided in Section II.)

- PC-12 cells (ATCC# CRL 1721)
- CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay (Cat.# G5421)
- complete medium
- trypsin/EDTA
- Mg^{2+}- and Ca^{2+}-free Dulbecco's PBS (DPBS)
- rat tail collagen type I in DPBS
- NGF diluent
- 96-well plates

A. Protocol

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

1. 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 2×10^6 cells per flask.
2. 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.
3. 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.
4. Pellet the cells by centrifugation (5 minutes at $250 \times g$) and wash once with 10ml of serum-free RPMI medium (37°C).
5. Resuspend the cells in 10ml of serum-free RPMI medium (37°C), count them and then suspend at a final density of 4×10^5 cells/ml.
6. 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.
7. Seed 36 wells with 3.6×10^4 cells/well (90µl/well). Incubate cells at 37°C in a humidified 5% CO_2 atmosphere while preparing the 2.5S mNGF samples.
8. 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.
9. Add 10µl of each 2.5S mNGF dilution to the test wells (in triplicate) containing the plated cells (final volume = 100µl/well).
10. Incubate the plate for 20 hours at 37°C in a humidified 5% CO_2 atmosphere.
11. Add 20µl/well of freshly prepared combined MTS/PMS solution.
12. Incubate the plate for 5 hours at 37°C in a humidified 5% CO_2 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.
13. Record the absorbance at 490nm using an ELISA plate reader.
14. Plot the absorbance at 490nm (Y axis) versus concentration of growth factor (X axis) and determine the ED_{50} 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 supplemented 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_2PO_4
1.15g	Na_2HPO_4

NGF diluent (prepare fresh)
RPMI 1640 medium supplement to contain a final concentration of:

100µg/ml	BSA

Filter-sterilize (0.22µm pore) and use immediately.

III. Related Products

Product	Size	Cat.#
CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay	1,000 assays	G5421
	5,000 assays	G5430
	50,000 assays	G5440

IV. References

1. Bocchini, V. and Angeletti, P.U. (1969) The nerve growth factor: Purification as a 30,000-molecular-weight protein. *Proc. Natl. Acad. Sci USA* **64**, 787–94.
2. Robinson, J.C. and Stammers, R. (1994) An in vitro bioassay for nerve growth factor based on 24-hour survival of PC-12 cells. *Growth Factors* **10**, 193–6.
3. Fonnum, F. (1975) A rapid radiochemical method for the determination of choline acetyltransferase. *J. Neurochem.* **24**, 407–9.

V. Summary of Changes

The 1/19 version of this Certificate/Protocol has been revised to add Quality Control Specifications on p. 1, and to update the reference list on p. 2.

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