

Mouse NGF/NGF β ELISA Kit

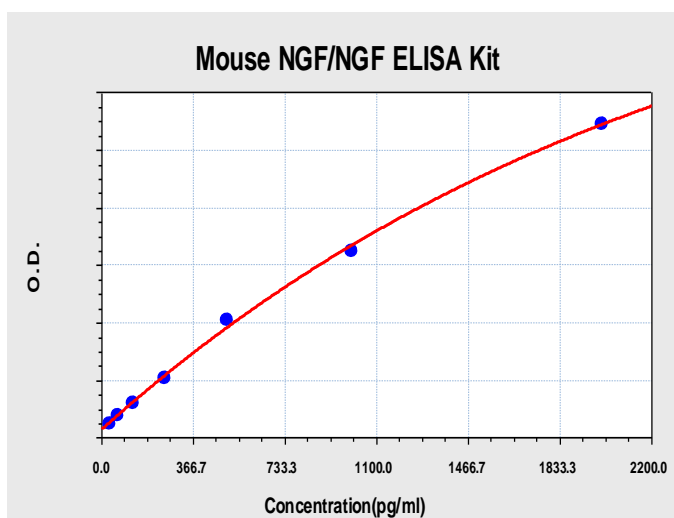
Typical Data Obtained from Mouse NGF

(TMB reaction incubate at 37°C for 25 min)

Concentration	0.0pg/ml	31.2pg/ml	62.5pg/ml	125pg/ml	250pg/ml	500pg/ml	1000pg/ml	2000pg/ml
O.D	0.052	0.121	0.194	0.298	0.571	0.973	1.641	2.362

Typical Mouse NGF ELISA Kit Standard Curve

This standard curve was generated for demonstration purpose only. A standard curve must be run with each assay.



Range	31.2 pg/ml-2000 pg/ml
Sensitivity	<1 pg/ml
Specificity	Natural recombinant mouse NGF
Cross-Reactivity	No detectable cross-reactivity with BDGF, GDNF, CNTF, Nt3 and NT4

Intra/Inter Assay Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

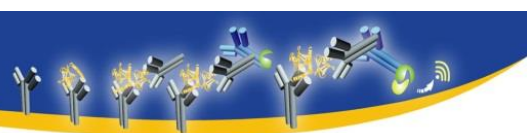
Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	136	727	1326	153	839	1645
Standard deviation	5.3	41.44	83.54	6.58	53.7	121.73
CV(%)	3.9	5.7	6.3	4.3	6.4	7.4

Assay Principle

GenWay Biotech, Inc.'s Mouse NGF/NGF Beta ELISA Kit was based on standard sandwich enzyme-linked immunosorbent assay technology. A monoclonal antibody from mouse specific for NGF/NGF Beta has been precoated onto 96-

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well plates. Standards(Expression system for standard: NSO; Immunogen sequence: S122-G241) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for NGF/NGF Beta is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the Mouse NGF/NGF Beta amount of sample captured in plate.

Kit Components

Description	Quantity
96-well plate precoated with anti-mouse NGF/NGF beta antibody	1
lyophilized recombinant mouse NGF/NGF beta standard	10ng/tubex2
biotinylated anti- mouse NGF/NGF beta antibody	130ul(dilution 1:100)
Avidin-Biotin-Peroxidase Complex(ABC)	130ul(dilution 1:100)
sample diluent buffer	30ml
antibody diluent buffer	12ml

Preparation

• Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- **Cell culture supernate, tissue lysate or body fluids:** Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C
- **Serum:** Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 2000 X g for 20 min. Analyze the serum immediately or aliquot and store frozen at -20°C.

• Sample Dilution Guideline

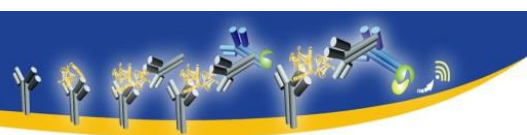
The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be well mixed with the diluents buffer.**

- **High target protein concentration (20-200ng/ml).** The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.
- **Medium target protein concentration (2-20ng/ml).** The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.
- **Low target protein concentration (31.2-2000pg/ml).** The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.
- **Very Low target protein concentration (≤31.2pg/ml).** No dilution necessary, or the working dilution is 1:2.

• Reagent Preparation and Storage

A. Reconstitution of the mouse NGF standard: NGF standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of NGF standard (10ng per tube) are included in each kit. Use one tube for

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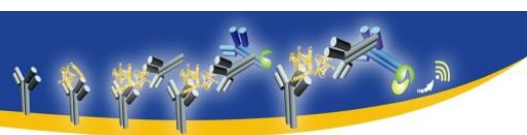


each experiment.

- a. 10,000pg/ml of mouse NGF standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
- b. 2000pg/ml of mouse NGF standard solution: Add 0.2 ml of the above 10ng/ml NGF standard solution into 0.8 ml sample diluent buffer and mix thoroughly.
- c. 1000pg/ml→31.2pg/ml of mouse NGF standard solutions: Label 6 Eppendorf tubes with 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 2000pg/ml NGF standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 10ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

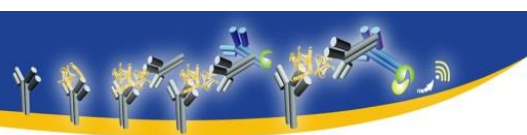
- B. Preparation of biotinylated anti-mouse NGF antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.
 - a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
 - b. Biotinylated anti-mouse NGF antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.
- C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.
 - a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
 - b. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly.



Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard NGF detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of NGF amount in samples.

1. Aliquot 0.1ml per well of the 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml mouse NGF standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Zero well). Add 0.1ml of each properly diluted sample of mouse serum, body fluids, tissue lysates or cell culture supernatants to each empty well. **See “Sample Dilution Guideline” above for details.** We recommend that each mouse NGF standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-mouse NGF antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash plate 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
(**Plate Washing Method:** Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)
6. Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
8. Add 90 µl of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 25-30 min (**Note:** For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated mouse NGF standard solutions; the other wells show no obvious color).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.



For calculation, (the relative O.D.₄₅₀) = (the O.D.₄₅₀ of each well) – (the O.D.₄₅₀ of Zero well). The standard curve can be plotted as the relative O.D.₄₅₀ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The mouse NGF concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Summary

1. Add samples and standards and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C in dark for 25-30 min.
5. Add TMB stop solution and read.



GenWay Biotech, Inc.
Protein and Antibody Solutions
6777 Nancy Ridge Drive
San Diego, CA 92121

Phone: 858-458-0866
Fax: 858-458-0833

sales@genwaybio.com
www.genwaybio.com

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