(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

NGF (Rat) ELISA Kit Manual

Cat No.: ER10798

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

Email (Order) order@enkilife.com

Email (Techsupport) techsupport@enkilife.com

Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Product description

This ELISA kit applies to the in vitro quantitative determination of NGF concentrations in serum, plasma and other biological fluids.

Key Features

●Sensitivity：23.43pg/mL.
●Detection range：39.62-2500pg/mL.
●Specificity：It can detect NGF, in samples without obvious cross-reaction with other analogs
●Repeatability：The coefficient of variation within and between plates is <10%.

Test Principle:

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Rat NGF. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Rat NGF and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Rat NGF, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Rat NGF. You can calculate the concentration of Rat NGF in the samples by comparing the OD of the samples to the standard curve.

Components and storage

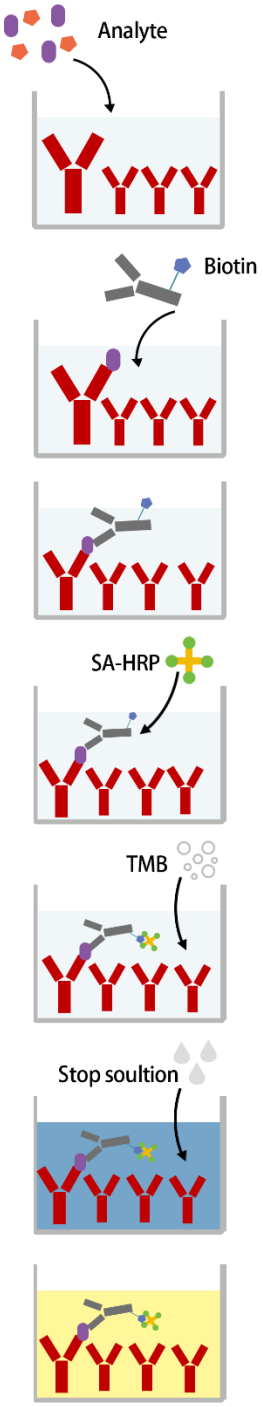
Store the product at 2-8°C before unopened. Upon receipt, unpack promptly and store as recommended in the instructions.

Components	Specifications	Storage and Notes	
Micro Plate	96T:8 wells×12 strips	**Unopened:** -20℃, 12 months	**Unused:** Put it back in the aluminum foil bag and seal it, store it at -20℃
	48T:8 wells×6 strips		
Reference Standard	96T: 2 vials	**Undissolved:** -20℃, 12 months	Please use freshly dissolved standards for

	48T: 1 vial		each experiment. Discard any unused standards after dissolution
Biotinylated Detection Ab Concentrate (100×)	96T: 120μL×1 vial	-20℃, 12 months	**Unused:** Please seal the concentrate and store it at -20℃, and discard the working solution
	48T: 60μL×1 vial		
HRP Conjugate Concentrate (100×)	96T: 120μL×1 vial	-20℃(Protect from light), 12 months	**Unused:** Please seal the concentrate and store it at -20℃, and discard the working solution
	48T: 60μL×1 vial		
Reference Standard & Sample Diluent	20mL×1	2-8℃, 12 months	
Biotinylated Detection Ab Diluent	14mL×1		
HRP Conjugate Diluent	14mL×1		
Wash Buffer Concentrate (25×)	30mL×1		
Substrate Reagent(TMB)	10mL×1	2-8℃(Protect from light),12 months	
Stop Solution	7mL×1	2-8℃/Room temperature	
Plate Sealer	5 pieces		
Product manual	1 copy		
Certificate of Analysis	1 copy		

Note:
Please ensure that the caps of all reagent bottles are tightened to prevent reagent evaporation and avoid microbial contamination.
The real volume will be slightly larger than the volume indicated on the label. Please use accurate measuring equipment instead of pouring directly.

Assay Procedures



1.Add 100μL standard or sample to each well, incubate at 37°C for 90 min.

2.Aspirate each well and wash with Wash Buffer, add 100μL Biotinylated Detection Ab working solution to each well. Incubate at 37°C for 60 min.

3.Aspirate and wash the plate for 3 times.

4.Add 100μL HRP conjugate working solution. Incubate at 37°C for 30 min. Aspirate and wash the plate for 5 times.

5.Add 90μL Substrate Reagent. Incubate at 37°C for 15 min.

6.Add 50μL Stop Solution.

7.Readings at 450nm. Calculation of results.

Operation method

ⓘ Precautions

1. Please wear protective equipment during the experiment. Please pay attention to biosafety protection according to the Biological Laboratory Safety Protection Regulations when contacting blood samples or other biological samples.
2. Kit components of different batches cannot be mixed (except Stop Solution).
3. The EP tubes and pipette tips used in the experiment are disposable, please do not mix

them.
4. There may be a small amount of water-like substances in the wells of the newly opened ELISA plate. This is a normal phenomenon and will not affect the experimental results. Temporarily unused strips should be placed in a spare aluminum foil bag and stored according to the required storage conditions.
5. Do not reuse the diluted standard, biotinylated antibody working solution, and enzyme conjugate working solution.
6. Unused Biotinylated Detection Ab Concentrate (100×), HRP Conjugate Concentrate (100×) and other original solutions should be stored according to the required storage conditions.
7. The ELISA reader needs to be equipped with a filter that can detect a wavelength of 450±10nm, and the detection range is between 0-3.5.

Materials required but not supplied

1. Microplate reader (450nm wavelength filter), Incubator capable of maintaining 37℃.
2. 1.5mL EP tube, absorbent paper.
3. Pipette and disposable tip: 0.5-10μL, 2-20μL, 20-200μL, 200-1000μL.
4. Double distilled water or deionized water.

Preparation

1. Equilibrate to room temperature: Take the Elisa kit out of the refrigerator 20 minutes in advance and let it equilibrate to room temperature.
2. Preheat the microplate reader: Please turn on the microplate reader at least 15 minutes in advance to stabilize the light source of the microplate reader during experiment.
3. Washing solution: Dilute the Wash Buffer Concentrate (25×) with double distilled water (1:24).Tip: The Wash Buffer Concentrate (25×) taken out of the refrigerator may have crystals, which is a normal phenomenon. You can use a 40℃ water bath to slightly heat it to completely dissolve the crystals before preparing the washing solution. Use it on the same day.
4. Standard working solution:
4.1 Centrifuge the standard at 10000×g for 1 minute.
4.2 Add 1.0 mL of Reference Standard &Sample Diluent to the freeze-dried standard. After tightening the tube cap, let it stand for 10 minutes and invert it gently several times.
4.3 After the standard is completely dissolved, gently mix with a pipette to prepare a 2500pg/mL standard working solution.

4.4 According to the experimental requirements, the standard is diluted in multiples. The recommended concentration gradient is: 2500、1250、625、312.5、156.25、78.13、39.65、0 pg/mL。

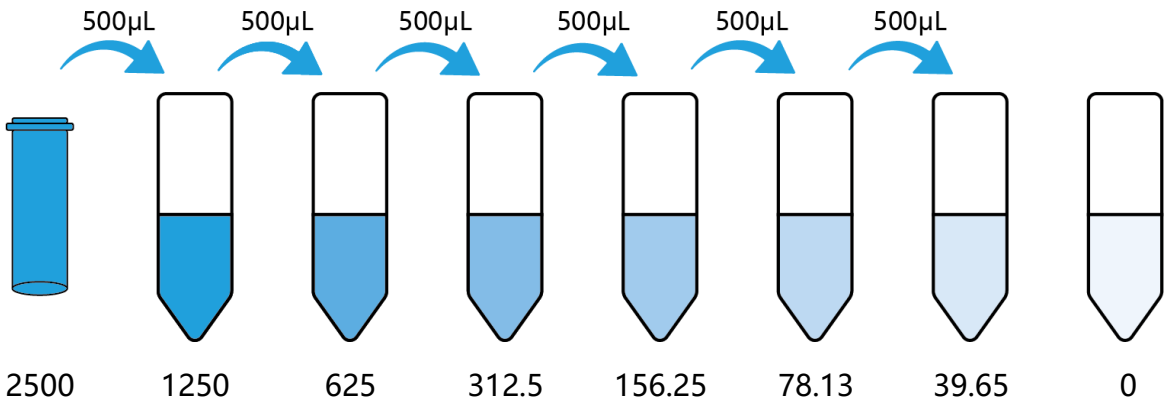
4.5 Multiple dilution method:

Prepare 7 EP tubes and add 500μL of Reference Standard & Sample Diluent to each tube. Pipette 500μL of the 2500pg/mL standard working solution and add it to the first EP tube. Mix it with a pipette to prepare a 1250 pg/mL standard working solution.

Next, take 500μL of the 1250 pg/mL EP tube and add it to the second EP tube. Mix it with a pipette to prepare a 625 pg/mL standard working solution. And so on to obtain a standard working solution with multiple dilutions.

Note: The last tube is regarded as a blank. Just add the Reference **Standard & Sample Diluent.**

The illustration as below is for reference:



5. Biotinylated Detection Ab working solution:

Calculate the amount: Calculate based on the reagent requirement of 100μL/well, and add an additional 100-200μL in case of emergency.

Antibody dilution:

15 minutes before the experiment, use Biotinylated Detection Ab Diluent to dilute and mix **Biotinylated Detection Ab Concentrate (100×)** to 1× working concentration. The diluted antibody should be used on the same day to ensure the experimental effect.

6. HRP Conjugate working solution:

Calculate the amount: Calculate based on the reagent requirement of 100μL/well, and add an additional 100-200μL in case of emergency.

Antibody dilution:

15 minutes before the experiment, use Biotinylated Detection Ab Diluent to dilute and mix **HRP Conjugate Concentrate (100×)** to 1× working concentration. The diluted antibody

should be used on the same day to ensure the experimental effect.

Operation Steps

1. Add standard and sample:

Add different concentrations of **Standard working solution** to the first two columns of ELISA plate wells from top to bottom, two wells for each concentration, 100μL per well; Add 100μL of the sample to each of remaining wells. High concentration samples need to be diluted first.
During operation, solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall, shake gently to mix, avoid bubbles, and complete the sample addition operation within 10 minutes.

2. Cover the plate and incubate:

Cover the plate with the **Plate Sealer** provided in the kit. Incubate at 37℃ for 90min.

3. Biotinylated Detection Ab working solution:

Tear off the sealer, remove and dry the liquid in the plate wells, no need to wash.
Add 100μL of **Biotinylated Detection Ab working solution** to each well. Shake gently to mix, and cover the plate with a new **Plate Sealer**. Incubate at 37℃ for 60min.

4. Wash plate 1:

Remove and dry the liquid in the plate wells, add 350μL of **Washing solution** to each well, Soak for 1-2min, remove and dry the liquid in the plate wells, pat it dry against clean absorbent paper, and complete one wash.
Repeat this wash step 3 times. Dry the liquid in the plate wells for the next step.

5. Add HRP Conjugate working solution:

Add 100μL of **HRP Conjugate working solution** to each well. Gently shake to mix, cover the plate with a new **Plate Sealer**. Incubate at 37℃ for 30min.

6. Wash plate 2:

Wash the plate 5 times, the steps are the same as step 4(**Wash plate 1**).

7. Add Substrate Reagent(TMB):

Add 90μL of **Substrate Reagent(TMB)** to each well. Cover the plate with a new **Plate Sealer**. Incubate at 37℃ for about 15min, Protect the plate from light.
Tips: Adjust the incubation time according to the color change, but do not exceed 30 minutes. Once the standard wells show a clear gradient, the incubation can be stopped.

8. Stop the reaction:

Add 50μL of **Stop Solution** to each well to stop the reaction.
Tip: The order of adding Stop Solution should be as consistent as possible with the order of adding Substrate Reagent(TMB).

9. Measure the OD value:

Immediately measure the OD value (optical density) of each well of the ELISA plate with a micro-plate reader set to 450nm.

Calculation of results

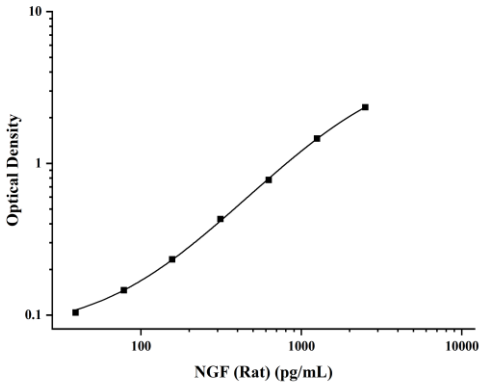
1. Calculate the average OD value and draw a standard curve:
Calculate the average OD value of each set of standard duplicate wells.
Subtract the OD value of the blank well from the average OD value of each standard to obtain the corrected OD value of the standard.
Use the concentration as the x-axis and the OD value as the y-axis to draw the standard curve using the Logistic function. Exclude the OD value of the blank group when drawing.
2. Calculation of sample concentration:
Substitute the OD value measured by the sample into the curve to find the concentration corresponding to the sample OD value. If the sample has a dilution multiple, multiply the obtained concentration by the dilution multiple to obtain the sample original solution concentration.
3. Processing of high OD value samples:
If the OD of the sample surpasses the upper limit of the standard curve, the sample should be appropriately diluted and re-measured.

Technical Data

Typical Data:

The following data is for reference only. A standard curve must be run with each assay.

Concentration: pg/mL	2500	1250	625	312.5	156.25	78.13	39.65	0
OD	2.345	1.457	0.778	0.43	0.233	0.146	0.104	0.058
Corrected OD	2.287	1.399	0.72	0.372	0.175	0.088	0.046	-



Precision:

Intra-assay precision (Precision within an assay): Low, mid range and high levels of the samples were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): Low, mid range and high levels of the samples were tested on 3 different plates, 20 replicates in each plate, respectively.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	132.54	248.52	1035.41	133.01	257.4	1007.36
Standard deviation	6.72	12.95	52.6	6.78	11.56	32.44
CV (%)	5.07	5.21	5.08	6.78	4.49	3.22

Recovery:

5 different levels of target protein spiked in samples were evaluated for recovery experiments to determine the range of recovery and calculate the average recovery rate.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=5)	86-99	92
EDTA plasma (n=5)	89-102	96
Cell culture media (n=5)	91-108	98

Linearity:

5 different levels of target protein spiked in samples were evaluated for recovery experiments to determine the range of recovery and calculate the average recovery rate. Subsequently, the 5 samples were diluted to 2-fold, 4-fold, 8-fold, and 16-fold

concentrations, and recovery experiments were performed again to evaluate the range and average recovery rate at different dilution factors.

		Serum (n=5)	Plasma (EDTA)(n=5)	Cell culture media (n=5)
1:2	Range (%)	87-100	93-108	92-104
	Average (%)	94	101	97
1:4	Range (%)	85-98	95-111	90-104
	Average (%)	91	102	97
1:8	Range (%)	92-102	96-107	93-105
	Average (%)	97	102	97
1:16	Range (%)	87-98	96-107	92-106
	Average (%)	92	102	99

ⓘ Troubleshooting

If the results are not good enough, please take pictures and save the experimental data in time. Keep the used plate and remaining reagents. Then contact our technical support to solve the problem. Meanwhile, you could also refer to the following materials:

Problem	**Causes**	**Solutions**
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by gentle mixing
	Wells are not completely aspirated	Completely aspirate wells in between steps
Low signal	Insufficient incubation time	Ensure sufficient incubation time
	Incorrect assay temperature	Use recommended incubation temperature
	Inadequate reagent volumes	Check pipettes and ensure correct preparation
	Improper dilution	Check dilution steps
	HRP conjugate inactive or TMB failure	Mix HRP conjugate and TMB, rapid coloring

Low value	Plate reader setting is not optimal	Verify the wavelength and filter setting on the Microplate reader(450nm)
		Open the Microplate Reader ahead to pre-heat at least 15 min
Large CV	Inaccurate pipetting	Check pipettes.
High background	Concentration of target protein is too high	Use recommended dilution factor
	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed. Ensure to use the wash buffer supplied in the Elisa kit
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions
	Stop solution is not added	Stop solution should be added to each well before measurement.

Sample processing:

ⓘ Sample collection

1.Serum: The whole blood sample is placed at room temperature for 2 hours or at 4°C overnight, centrifuge it at 1000×g for 20 min and collect the supernatant to carry out the assay. The tube used to collect blood should be a disposable endotoxin-free tube.
2.Plasma: Collect plasma using EDTA-Na2 as an anticoagulant. Centrifuge samples for 15 min at 1000×g within 30 min of collection. Collect the supernatant to carry out the assay. Avoid using hemolyzed or hyperlipidemia samples.
3.Tissue homogenization: Rinse the tissue with pre-cooled PBS (0.01M, pH=7.4) to remove residual blood, weigh and mince the tissue into small pieces. Add the minced tissue and the corresponding volume of PBS (generally, tissue weight (g): PBS (mL) volume=1:9, eg. adding 1g of tissue sample to 9mL of PBS, the specific ratio can be adjusted appropriately according to experimental needs. It is recommended to add protease inhibitors to PBS) into a glass homogenizer and grind thoroughly on ice. To further break down the cells, the homogenate can be ultrasonically broken or repeat

freeze-thaw cycles. The homogenates are then centrifuged for 5-10 minutes at 5000×g , and collect the supernatant to carry out the assay.

4.Cell lysates:

Adherent cells: Gently wash with pre-cooled PBS, then digest with trypsin, and collect cells after centrifugation for 5 minutes at 1000×g.

Suspended cells: The cells can be collected directly by centrifugation.

The collected cells are washed 3 times with pre-cooled PBS. Add 150-200μL PBS to each 10^6 cells, resuspend and break the cells by repeating freeze-thaw process (if the content is very low, the volume of PBS can be reduced). Centrifuge for 10 minutes at 1500×g and collect the supernatant to carry out the assay.

5.Cell culture supernatant or other biological fluids: Centrifuge samples for 20 min at 1000×g, remove impurities and cell debris. Collect the supernatant to carry out the assay.

ⓘNote for samples

1. Sample storage after collection:

Assay within 1 week: store at 2-8℃.

Assay within 1 month: please divide into single-use amounts and store at -20℃.

Assay within 3 months: please divide into single-use amounts and store at -80℃.

2.Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, it is recommended to conduct a preliminary experiment to determine the appropriate dilution ratio to ensure the accuracy of the results. (It is recommended to conduct a preliminary experiment after consulting the literature to determine the dilution multiple)

3. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity

4.If a lysis buffer is used to prepare tissue homogenates or cell lysates, there is a possibility of causing a deviation due to the introduced chemical substance.

5. Please be aware that some recombinant proteins may not be detected due to a mismatching with the coated antibody or detection antibody.

ⓘStatement:

1. **Quality and Technical Risk Tips**: Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.

2. **Factors affecting experimental results:** The experimental results are affected by many factors such as the effectiveness of the reagents, the skills of the operator and the experimental environment. To ensure the accuracy of the results, please prepare sufficient samples to be tested.

3. **Reagent Usage Guide:** To obtain the best experimental results, it is recommended to only use the reagents provided in this kit and operate strictly in accordance with the instructions. Avoid mixing with products from other manufacturers.

4. **Operation Precautions:** Improper reagent preparation or microplate reader parameter settings may lead to abnormal experimental results. Please read the instructions carefully before the experiment and adjust the instrument parameters correctly.

5. **Result Reproducibility:** Even the same operator may get different results in two independent experiments. To ensure the reproducibility of the results, please strictly control every step of the operation during the experiment.

6. **Quality Assurance and Difference Description:** Every kit has strictly passed QC test. However, results from users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipment, and so on. Intra-assay variance among kits from different batches might arise from the above reasons too.

We are always committed to providing high-quality products and thank you for your understanding and support. If you have any questions, please feel free to contact our technical support team.