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Sunlong Medical™ Camel Noradrenaline (NE) ELISA Kit

Cat No.:EL0099Cm

Manual

Type:	Competitive ELISA	Detection Range:	0.625-40μg/mL
Species:	Pan-species	Storage Temp.:	2-8℃
Format:	96T/48T	Shelf Life:	6 months
Intended Use: This product is for research use only. It is intended for the quantitative detection of Noradrenaline in cell culture supernatant, serum, plasma ,and other biological fluids.			

Assay Principle

This kit uses an indirect Competitive ELISA method. The microplate wells are pre-coated with a conjugated antigen. Noradrenaline in the sample competes with the pre-coated antigen for binding to the Noradrenaline antibody. After adding the Enzyme-labeled Secondary Antibody, a coated antigen–antibody–enzyme conjugate complex is formed. The enzyme then catalyzes the TMB substrate to produce a color reaction. The absorbance of the sample is inversely proportional to the Noradrenaline concentration. A standard curve is used to quantify the Noradrenaline content in the sample. The calculated value is multiplied by the dilution factor to obtain the actual concentration.

Precautions

1. **This kit is for research use only. Not for use in diagnostic procedures.**
2. After opening the sealed bag containing the pre-coated strips, any unused strips should be immediately resealed in a ziplock bag with desiccant. To minimize inter-assay variation, strips can be stored at 2-8°C if the next assay is within 48 hours. For longer intervals, store at -20°C and re-run the standard curve in the next assay.
3. The Concentrated Wash Buffer, Concentrated Primary Ab, and Concentrated HRP Conjugate must be diluted as instructed before use. Prepare these solutions as needed on the day of use.

4. It is recommended to mix Substrate Solution A and Substrate Solution B in a disposable tube first before adding to the wells. Prepare the mixed substrate solution immediately before use. The prepared substrate solution should remain colorless until added to the plate.
5. **The Stop Solution provided in this kit is a dilute sulfuric acid solution, which is corrosive. Handle with care. If contact with skin occurs, wipe off immediately and rinse thoroughly with water.**
6. **Operate strictly according to the instructions. If in doubt, please confirm with technical support before proceeding to avoid waste of samples and time.**
7. Do not mix reagents from different lot numbers. Do not use reagents from other sources or manufacturers.
8. Seal plates, absorbent paper, EP tubes, and pipette tips used during sample addition are for single use only. Do not reuse.

Kit Components

Component	96T Config	48T Config	Storage Condition
Pre-coated Plate	8 wells × 12 strips	8 wells × 6 strips	2-8℃
Standard	1 vial × 200µL	1 vial × 100µL	
100× Primary Ab	1 vial × 100µL	1 vial × 50µL	
100× HRP-Conjugated Ab	1 vial × 100µL	1 vial × 50µL	
20× Dilution Buffer	1 bottle × 25mL	1 bottle × 15mL	
Substrate A	1 bottle × 6mL	1 bottle × 3mL	
Substrate B	1 bottle × 6mL	1 bottle × 3mL	
Stop Solution	1 bottle × 6mL	1 bottle × 3mL	
20× Wash Buffer	1 bottle × 25mL	1 bottle × 15mL	
Plate Sealer	4 sheets	4 sheets	RT
Instruction	1 copy	1 copy	

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Materials Required but Not Provided:

Equipment & Instruments	Other Materials
Microplate reader with 450 nm filter	Absorbent paper or lab wipes
Pipettes of various volumes	Distilled or deionized water
Incubator capable of maintaining 37°C	Various pipette tips and EP tubes

Sample Collection and Handling:

1. **Serum:** Collect whole blood into serum separation tubes. Allow clotting at room temperature for 30 minutes to 2 hours. Centrifuge at 2-8°C, 2500-3500 × g for 20 minutes. Carefully collect the supernatant.
2. **Plasma:** Use EDTA or citrate as anticoagulant as required. Centrifuge samples within 30 minutes of collection at 2-8°C, 3000 × g for 15 minutes. Carefully collect the supernatant. If precipitation occurs during storage, re-centrifuge.
3. **Urine:** Collect using sterile tubes. Centrifuge at 2-8°C, 2500-3500 × g for 10 minutes. Carefully collect the supernatant. If precipitation occurs during storage, re-centrifuge. Handle pleural fluid, ascitic fluid, and cerebrospinal fluid similarly.
4. **Cell Culture Supernate:** Collect the fluid and centrifuge at 2-8°C, 2500-3500 × g for 20 minutes to remove impurities and cell debris. Collect the supernatant for assay.
5. **Cell Lysate:** Wash adherent cells gently with ice-cold PBS (0.01M, pH=7.4), then digest with trypsin. Collect cells by centrifugation at 2-8°C, 1000 × g for 5 minutes. Suspension cells can be collected directly by centrifugation. Wash collected cells 3 times with cold PBS. Resuspend 1×10^6 cells in 150-200μL PBS. Lyse cells by repeated freeze-thaw cycles or ultrasonication (it is recommended to add protease inhibitors to PBS; if the target concentration is expected to be low, reduce the PBS volume). Centrifuge the lysate at 2-8°C, 10000 × g for 10 minutes. Collect the supernatant for assay.
6. **Tissue Samples:** Rinse the tissue with ice-cold PBS (0.01M, pH=7.4) to remove residual blood. Weigh and mince the tissue. Add the minced tissue to an appropriate volume of PBS (generally a 1:9 weight/volume ratio, e.g., 1g tissue to 9mL PBS; the volume can be adjusted according to experimental needs and should be recorded. It is recommended to add protease inhibitors to PBS) in a homogenizer. Grind thoroughly on ice. To further lyse cells, subject the homogenate to repeated freeze-thaw cycles or ultrasonication. Finally, centrifuge the homogenate at 2-8°C, 10000 × g for 5-10 minutes. Collect the supernatant for assay.
7. **Other Biological Samples:** Centrifuge at 2-8°C, 2500-3500 × g for 20 minutes. Carefully collect the supernatant.

Sample Appearance: Samples should be clear and transparent. Suspended matter should be removed by centrifugation.

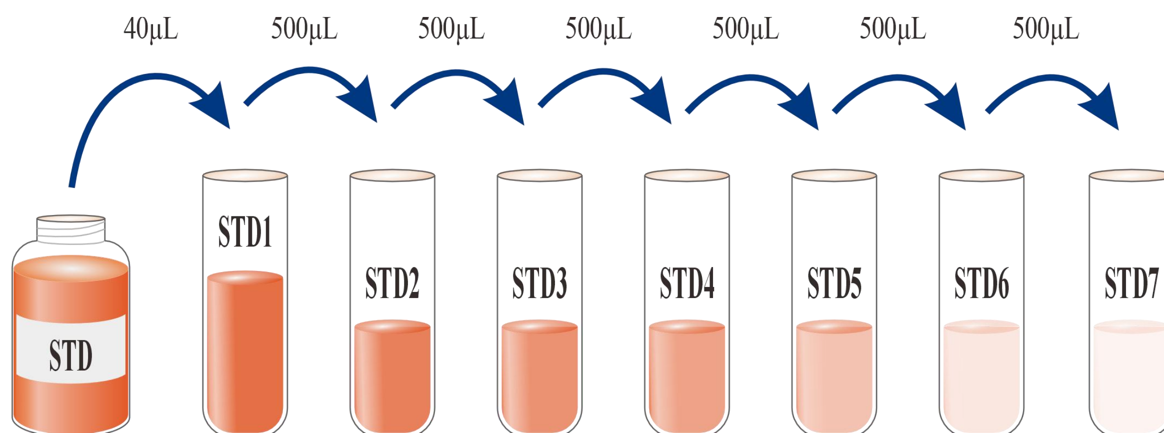
Sample Storage: Assay samples as soon as possible after collection. If testing cannot be performed immediately, aliquot the samples and store at -20°C (test within 1 month) or -80°C (test within 6 months). Avoid repeated freeze-thaw cycles.

Reagent Preparation

Allow all reagents to equilibrate to room temperature for about 30 minutes before use.

Wash Buffer/Diluent Preparation: If crystals have formed in the concentrated (20×) Wash Buffer/Diluent, warm it at 37°C until all crystals are completely dissolved. Dilute 1:20 with distilled water (e.g., add 1 mL of concentrated Wash Buffer to 19 mL of distilled water).

Standard Preparation: Take the standard from the kit and prepare 7 test tubes. First, draw a required amount of the 1 mg/mL standard (200 µL) and dilute it with 1× dilution buffer to 40 µg/mL (for example: 40 µL of the standard stock solution + 960 µL of 1× dilution buffer to prepare 1000 µL of a 40 µg/mL standard). Then, add 500 µL of 1× dilution buffer to each of 6 test tubes. Perform a serial 2-fold dilution of the 40 µg/mL standard in these 6 separate test tubes to create 6 gradients, resulting in a total of 7 standard concentrations: 40 µg/mL, 20 µg/mL, 10 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, and 0.625 µg/mL. Transfer 500 µL of the standard from the highest concentration solution to the next test tube, mix gently by pipetting, and continue the serial dilution in this manner (as shown in the figure). The 1× dilution buffer is used as the zero-concentration standard (0 µg/mL).



Concentrations after dilution are as follows (Unit: µg/mL)

STD (Stock)	STD1	STD2	STD3	STD4	STD5	STD6	STD7
1mg/mL	40	20	10	5	2.5	1.25	0.625

Primary Ab Working Solution Preparation(1×): 10minutes before use, centrifuge the 100× Primary Ab at 1000 × g for 1 minute. Then dilute the 100× Primary Ab with 1× Diluent to prepare the 1× Primary Ab working solution. Prepare this working solution on the day of use according to the required volume.

HRP-Conjugated Ab Working Solution Preparation(1×): 10minutes before use, centrifuge the 100× HRP-Conjugated Ab solution at 1000 × g for 1 minute. Then dilute the 100× HRP-Conjugated Ab with 1× Diluent to prepare the 1× HRP-Conjugated Ab working solution. Prepare this working solution on the day of use according to the required volume.

Note: If the Noradrenaline concentration in the sample is higher than the highest standard point, dilute the sample with an appropriate dilution factor as needed and re-assay. Multiply the result by the dilution factor.

Assay Procedure

All standards and samples are recommended to be assayed in duplicate.

1. **Microplate Preparation:**
   Determine the number of strips required for the assay. Remove other unused strips and immediately return them to the sealed bag containing desiccant.
2. **Sample and Primary Ab Incubation:**
   Add 50µL of different concentration standards / pre-treated samples to each well. Simultaneously add 50µL of Primary Ab Working Solution per well (use a multichannel pipette when adding the Primary Ab Working Solution). Cover with the plate sealer and incubate at 37°C for 30 minutes. After incubation, add 300µL of 1× Wash Buffer to each well, shake gently for 30 seconds, aspirate, and blot dry on absorbent paper. Repeat this wash process 3 times.
3. **HRP-Conjugated Ab Incubation:**
   Add 100µL of HRP-Conjugated Ab Working Solution to each well. Mix gently. Cover with the plate sealer and incubate at 37°C in the dark for 30 minutes. After incubation, repeat the washing procedure from Step 2, 4 times.
4. **Substrate Development:**
   Mix Substrate Solution A and Substrate Solution B in a 1:1 ratio to prepare the substrate working solution (prepare as needed; it should be colorless and transparent). Add 100µL

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of the prepared substrate working solution to each well. Cover with a new plate sealer. Incubate at 37°C protected from light for 15 minutes.

5. **Stop Reaction:**
   After the color development, add 50μL of Stop Solution to each well. Gently tap the plate to mix. Measure the Optical Density (OD) at 450 nm using a pre-warmed microplate reader within 5 minutes.

Calculation of Results

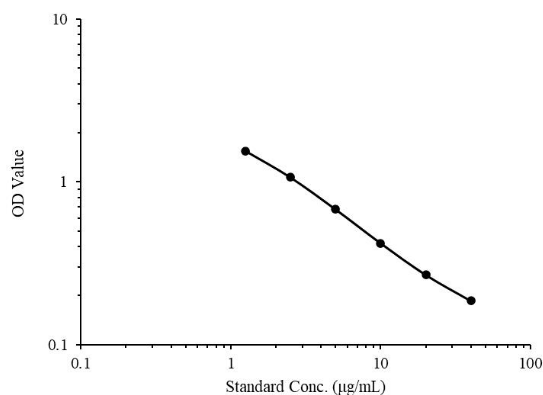
Plot the standard curve using the logarithm of the sample concentration as the X-axis and the logarithm of the OD value as the Y-axis, applying a four-parameter logistic function. Alternatively, use calculation software capable of generating a four-parameter logistic (4-P) curve fit to create the standard curve.

If the OD value of a sample is higher than the highest standard point, the sample should be appropriately diluted and re-assayed. Multiply the concentration obtained from the standard curve by the dilution factor.

Typical Data

The following data and curve are for reference only. The experimenter must establish a standard curve based on their own experimental data.

Standard Conc. (μg/mL)	40	20	10	5	2.5	1.25	0.625
OD Value	0.171	0.251	0.445	0.704	1.161	1.603	2.231



The standard curve shown in this figure is for example purposes only. Use the standard curve generated from the standards in the same assay to calculate sample results.

Precision

Intra-assay Precision: Three samples with known high, medium, and low concentrations were assessed twenty times within the same plate.
Intra-assay Coefficient of Variation (CV%) < 10%.

Inter-assay Precision: Three samples with known high, medium, and low concentrations were assessed twenty times in different plates.
Inter-assay Coefficient of Variation (CV%) < 15%.

Recovery

Sample recovery rate: 80% - 120%.

Sensitivity

Based on sample testing, the minimum detectable concentration of this kit is 0.625μg/mL.

Linearity

The correlation coefficient r value of the calibrator dose-response curve is greater than or equal to 0.998.

Specificity

This kit is specific for detecting Noradrenaline and has no significant cross-reactivity with other proteins.