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*Optimize Your Research*

# Human Neurotensin ELISA Kit

## USER INSTRUCTION

**Cat.No BTB-E1318Hu**

**Standard Curve Range**: 2pmol/L-600pmol/L

**Sensitivity**: 1.04pmol/L

**Size**: 96 wells

**Storage**: Store the reagents at 2-8°C. For long term storage refer to the expiration date keep it at -20°C. Avoid repeated thaw cycles.

**This product is for research use only, not for use in diagnosis procedures. It's highly recommend to read this instruction entirely before the use.*

## PRECICION

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.

CV(%) = SD/mean x 100

Intra-Assay: CV<8%

Inter-Assay: CV<10%

## INTENDED USE

This sandwich kit is for the accurate quantification of human Neurotensin ( also known as NT ) in serum, plasma, cell culture supernates, cell lysates, tissue homogenates.

## ASSAY PRINCIPLE

This kit is a Enzyme-Linked Immunosorbent Assay (ELISA). NT is added to the wells pre-coated with NT monoclonal antibody. After cubation a biotin-conjugated anti-human NT antibody is added and binds to human NT. After incubation unbound biotin-conjugated anti-human NT antibody is washed away during a washing step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human NT antibody. After incubation unbound Streptavidin-HRP is washed away during a

washing step. Substrate solution is then added and color develops in proportion to the amount of human NT. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

## REAGENT PROVIDED

| Components | Quantity |
|---|---|
| Standard Solution (640pmol/L) | 0.5ml |
| Pre-coated ELISA Plate | 12 * 8 well strips |
| Standard Diluent | 3ml |
| Streptavidin-HRP | 6ml |
| Stop Solution | 6ml |
| Substrate Solution A | 6ml |
| Substrate Solution B | 6ml |
| Wash Buffer concentrate (30x) | 20ml |
| Biotin-Conjugate Anti-human NT Antibody | 1ml |
| User Instruction | 1 |
| Plate Sealer | 2 |
| Zipper bag | 1 |

## MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37°C±0.5°C incubator
2. Microplate reader with 450 ± 10nm wavelength filter
3. Precision pipettes and disposable pipette tips
4. Clean tubes
5. Deionized or distilled water
6. Absorbent paper

## PRECAUTIONS

1. Prior to use, the kit and sample should be warmed naturally to room temperature 30 minutes.
2. Once the desired number of strips has been removed, immediately reseal the bag to protect the remain from deterioration. Cover all reagents when not in use.
3. Make sure pipetting order and rate of addition from well-to-well when pipetting reagents.
4. This instruction must be strictly followed in the experiment.

5. Pipette tips and plate sealer in hand should be clean and disposable to avoid cross-contamination.
6. Avoid using the reagents from different batches together.
7. Substrate solution B is sensitive to light, don't expose substrate solution B to light for a long time.
8. Stop solution contains acid. Please wear eye, hand and skin protection when using this material. Avoid contact of skin or mucous membranes with kit reagent.

## SPECIMEN COLLECTION

**Serum**: Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes.

**Plasma**: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 2000-3000 RPM at 2 - 8°C within 30 minutes of collection.

**Urine**: Collect by sterile tube. Centrifuge at 2000-3000 RPM for approximately 20 minutes. When collecting pleuroperitoneal fluid and cerebrospinal fluid, please follow the procedures above-mentioned.

**Cell culture supernatant**: Collect by sterile tubes when examining secrete components. Centrifuge at 2000-3000 RPM for approximately 20 minutes. Collect the supernatants carefully. When examining the components within the cell, use PBS (pH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells through repeated freeze-thaw cycles to let out the inside components. Centrifuge at 2000-3000 RPM for approximately 20 minutes.

**Tissue**: Rinse tissues in PBS(pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (pH7.4) with a glass homogenizer on ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2000-3000 RPM for approximately 20 minutes.

**Note**

1. Samples to be used within 5 days should be stored at 2-8°C. Samples should be aliquoted or must be stored at -20°C within 1 month or -80°C within 6 months. Avoid repeated freeze thaw cycles.

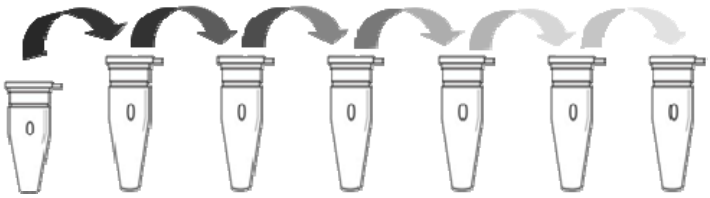
2. Samples should be brought to room temperature before starting the assay.
3. Samples containing NaN3 can't be tested as it inhibits the activity of Horse Radish Peroxidase (HRP).
4. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.
5. Hemolysis can greatly impact the validity of test results. Take care to minimize hemolysis.

***SAMPLE CAN'T BE DILUTED WITH THIS KIT. ONCE THE SAMPLE HAS BEEN DILUTED IT WILL RESULT IN HIGH BACKGROUND!***

## REAGENTS PREPARATION

1. All reagents should be brought to room temperature before use.
2. **Standard**: It is strongly recommended that all standards and samples be run in duplicate. Dilution of standard solutions suggested are as follows:

| | | |
|---|---|---|
| 320pmol/L | Standard No.5 | 120µl Original Standard + 120µl Standard diluent |
| 160pmol/L | Standard No.4 | 120µl Standard No.5 + 120µl Standard diluent |
| 80pmol/L | Standard No.3 | 120µl Standard No.4 + 120µl Standard diluent |
| 40pmol/L | Standard No.2 | 120µl Standard No.3 + 120µl Standard diluent |
| 20pmol/L | Standard No.1 | 120µl Standard No.2 + 120µl Standard diluent |



| Standard | S5 | S4 | S3 | S2 | S1 |
|---|---|---|---|---|---|
| 640pmol/L | 320pmol/L | 160pmol/L | 80pmol/L | 40pmol/L | 20pmol/L |

3. If the standard hasn't been run out, keep the remain at -20°C. Diluted standard can't be reused.
4. **Wash Buffer**: Dilute the wash buffer concentration (30x) with distilled water.
5. **Washing Method**: Discard the solution in the plate without touching the side. Blot the plate onto absorbent paper or other absorbent material. Soak each well with at least 0.35 ml wash buffer for 1~2 minutes.

## ASSAY PROCEDURE

1. Add 50μl standard to standard well.
2. Add 40μl sample to sample wells and then add 10μl anti-NT antibody to sample wells, then add 50μl streptavidin-HRP to sample wells and standard wells ( Not blank control well ) . Mix well. Cover the plate with a sealer. Shake gently to mix them up. Incubate 60 minutes at 37°C.
3. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells in wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.
4. Add 50μl substrate solution A to each well and then add 50μl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C at room temperature in the dark.
5. Add 50μl Stop Solution to each well, the blue color will change into yellow immediately.
6. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 30 min after adding the stop solution.

## SUMMARY

Prepare all reagents, samples and standards

⇩

Add samples, standards and ELISA solutions. Incubate for 60 minutes at 37°C

⇩

Wash the plate five times. Add substrate solution A and B. Incubate for 10 minutes at 37°C for color development

⇩

Add stop solution

⇩

Read the OD value within 10minutes.

⇩

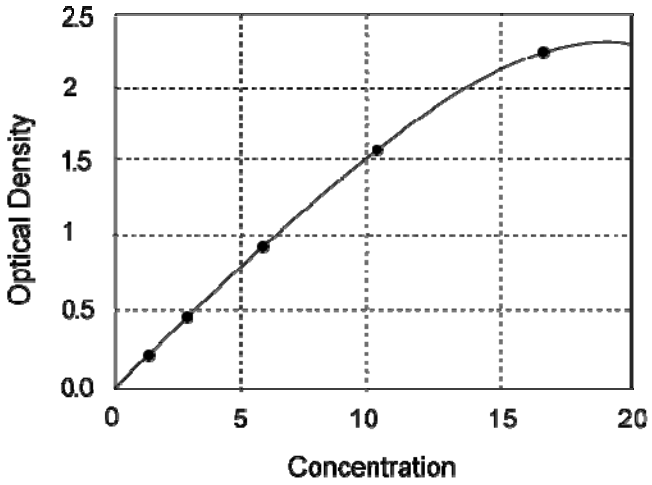
Calculate

## CALCULATION OF RESULTS

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis. If the standard have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated with each assay.



## TROUBLESHOOTING

| Possible Case | Solution |
|---|---|
| **High Background** | |
| • Improper washing<br>• Substrate was contaminated<br>• Non-specific binding of antibody<br>• Plate are not be sealing incompletely<br>• Incorrect incubation temperature<br>• Substrate exposed to light prior to use<br>• Contaminated wash buffer | • Increasing duration of soaking steps<br>• Replace.Substrate should be clean and avoid crossed contamination by using the sealer<br>• Replace another purified antibody or blocking buffer<br>• Make sure to follow the instruction strictly<br>• Incubate at room temperature<br>• Keep substrate in a dark place<br>• Use a clean buffers and sterile filter |
| **Weak Signal** | |
| • Improper washing<br>• Incorrect incubation temperature<br>• Antibody are not enough<br>• Reagent are contaminated<br>• Pipette are not clean | • Increasing duration of soaking steps<br>• Incubate at room temperature<br>• Increase the concentration of the antibody<br>• Use new one<br>• Pipette should be clean |
| **No Signal** | |
| • Reagent are contaminated<br>• Sample prepared incorrectly<br>• Antibody are not enough<br>• Wash buffer contains sodium azide<br>• HRP was not added | • Use new one<br>• Make sure the sample workable/dilution<br>• Increase the antibody concentration<br>• Use a new wash buffer and avoid sodium azide in it<br>• Add HRP according to the instruction |
| **Poor Standard Curve** | |
| • Improper standard dilution<br>• Inccorect storage of reagents<br>• Incomplete washing of the wells<br>• Capture antibody did not bind to the plate | • Reconstitute standard according to the instruction<br>• Store the reagents in the ELISA kit according to the printed instruction before using them<br>• Make sure wells are washed adequately by filling the wells with wash buffer and all residual antibody solutions crossed well before washing.<br>• Replace a new ELISA plate |

## DATA TEMPLATE

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | | | | | | | | | | | |
| B | | | | | | | | | | | | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

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