# Technical Manual

# Human Alzheimer-Associated Neuronal Thread Protein (AD7c-NTP) ELISA Kit

- Catalogue Code: HDES0092
- Antibody ELISA Kit
- Research Use Only

## 1. Test principle

This ELISA kit uses Sandwich-ELISA as the method to detect the Alzheimer-Associated Neuronal Thread Protein (AD7c-NTP) in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with AD7c-NTP monoclonal antibody. Samples and AD7c-NTP polyclonal antibody are sequentially added to the ELISA Microtiter plate wells, after wash, add HRP Conjugate. If AD7c-NTP present in the sample, it will combine with the pre-coated AD7c-NTP monoclonal antibody to form antibody-antigen-antibody- HRP Conjugate compound. Wash the Microtiter plate to remove unbound HRP Conjugates. The substrate reagent is added to initiate the color developing reaction. The presence of AD7c-NTP can be determined by measuring the absorbance value by using a microplate reader with 450 nm wavelength. The OD value is proportional to the concentration of AD7c-NTP. You can calculate the concentration of AD7c-NTP in the samples by comparing the OD of the samples to the standard curve.

## 2. Kit components

| Item | Specifications |
|---|---|
| ELISA Microtiter plate | 96 wells |
| Standard Liquid | 0.8 mL each<br>(0 ng/mL, 0.25 ng/mL, 1.0 ng/mL, 2.0 ng/mL, 5.0 ng/mL, 10 ng/mL) |
| 25×Concentrated Wash Buffer | 20 mL |
| HRP Conjugate | 5 mL×2 |
| Binding Antibody | 5 mL×2 |
| Substrate Reagent A | 5 mL |
| Substrate Reagent B | 5 mL |
| Positive Control | 0.8 mL |
| Negative Control | 0.8 mL |
| Stop Solution | 5 mL |
| Plate Sealer | 3 pieces |
| Sealed Bag | 1 piece |
| Manual | 1 copy |

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

## 3. Other materials required but not supplied

- Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
- High-precision transferpettor, EP tubes and disposable pipette tips

- 37°C Incubator or water bath
- Deionized water
- Absorbent paper

## 4. Notes

1. Please read the manual carefully before use, changes of operation may result in unreliable results.
2. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly executed. All the waste should be handled as contaminant.
3. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.
4. The ELISA Microtiter plate obtained from cold storage conditions should be adjusted to room temperature before use. The unused plate should be kept in a sealed bag with desiccant.
5. Concentrated washing liquid at low temperature condition is easy to crystallization, it should be adjusted to room temperature in order to dissolve completely before use.
6. The results shall depend on the readings of the micro-plate Reader.
7. **Each reagent is optimized for use in the HDES0092. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0092 with different lot numbers.**
8. Different batch numbers of reagents should not be mixed.
9. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.
10. The blood source raw material used in the preparation of the kit has been inactivated at 60°C, all of them were test by HBsAg, anti-HCV, anti-HIV, anti-TP test kits approved by the SFDA, and they present negative result, however there is no test method can completely guarantee absolute safety, furthermore, the sample is derived from human blood. Therefore both samples and kits should be treated as potentially infectious reagents.

## 5. Storage and expiry date

Store at 2-8°C. Avoid freeze.
Please store the opened plate at 2-8°C, the shelf life of the opened kit is up to 2 month under no corrosive gas enviro.
**Expiry date:** expiration date is on the packing box.

## 6. Sample preparation

1. Take a 24h urine sample or mid-stream urine from morning urine, mix well, and observe with the naked eye. If the urine is cloudy or dark in color, it should be discarded and a clear sample should be selected for measurement. Store at 2-8 °C
2. **Wash Buffer:** The **25×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:24.

## 7. Assay procedure

Restore all reagents and samples to room temperature (25°C) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8°C.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:** add 100 µL of **Standard or Sample** per well, set the blank control well.
3. **Incubate:** gently tap the plate to mix thoroughly. Cover the ELISA Microtiter plate with sealer. Incubate for 60 min at 37°C in shading light.
4. **Wash:** remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with **Wash Buffer** and immerse for 30-60 sec each time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **Add Binding Antibody:** add 100 µL of AD7C-NTP polyclonal Antibody per well, keep the blank control well empty.
6. **Incubate:** gently tap the plate to mix thoroughly. Cover the ELISA Microtiter plate with sealer. Incubate for 30 min at 37°C in shading light.
7. **Wash:** repeat step 4.
8. **Add HRP Conjugate:** add 100 µL of HRP Conjugate per well, keep the blank control well empty.
9. **Incubate:** gently tap the plate to mix thoroughly. Cover the ELISA Microtiter plate with sealer. Incubate for 30 min at 37°C in shading light.
10. **Wash:** repeat step 4.
11. **Add substrate:** add 50 µL of **Substrate Reagent A** and 50 µL of **Substrate Reagent B** to each well. Gently tap the plate to mix thoroughly. Cover with a new plate sealer. Incubate for 15 min at 37°C in shading light.
12. **Stop reaction:** add 50 µL of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
13. **OD Measurement:** set the Microplate Reader wavelength at 450 nm to detect A value of each well, zero set with blank well.

Note: If the A value of highest concentration standard (10 ng/mL) ≥0.8, the test result is valid.

## 8. Result analysis

1. Create a standard curve by plotting the absorbance of each standard on the y-axis against the concentration on the x-axis, and set double log fit to draw a standard curve. Add average absorbance value of sample to standard curve to get corresponding concentration.
2. If the A value of sample is higher than highest concentration standard (10 ng/mL), the sample should be diluted. And the concentration calculated from the standard curve must be multiplied by the dilution factor.

## 9. Reference value

Normal reference＜1.5 ng/mL.
Due to differences in geography, race, gender and age, it is recommended that each laboratory establish its own reference value (range).

## 10. Product performance

1. **Accuracy:** the recovery rate should be within 85-115%.
2. **Limit of detection:** should not be more than 0.25 ng/mL.
3. **Linear range:** in the range of [0.25 ng/mL, 10 ng/mL], the correlation coefficient r≥0.98.
4. **Repeatability:** CV ≤ 15% (n=10) .
5. **Inter-batch difference：** CV ≤ 20%.
6. **Specificity:** there is no cross-reactivity with phosphorylated tau protein, β42 and β40.

## 11. Limitations of this test method

1. This product can only be used for the determination of urine samples and can not be used for other body fluid samples.
2. To avoid interfering with the results, serum with high blood lipids and hemolysis should be avoided. Samples with obvious contamination should not be used for testing as they may lead to erroneous experimental results.

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