Human AD7C-NTP ELISA Kit

Cat. No.:DEIA1126
Pkg.Size:96T

Intended use

The Human AD7C-NTP ELISA kit is designed to detect and quantify the level of human AD7C-NTP in urine.

General Description

Although Alzheimer's disease develops differently for every individual, there are many common symptoms. Early symptoms are often mistakenly thought to be 'age-related' concerns, or manifestations of stress. In the early stages, the most common symptom is difficulty in remembering recent events. When AD is suspected, the diagnosis is usually confirmed with tests that evaluate behaviour and thinking abilities, often followed by a brain scan if available. As the disease advances, symptoms can include confusion, irritability and aggression, mood swings, trouble with language, and long-term memory loss. As the sufferer declines they often withdraw from family and society. Gradually, bodily functions are lost, ultimately leading to death. Since the disease is different for each individual, predicting how it will affect the person is difficult. AD develops for an unknown and variable amount of time before becoming fully apparent, and it can progress undiagnosed for years. On average, the life expectancy following diagnosis is approximately seven years. Fewer than three percent of individuals live more than fourteen years after diagnosis. The cause and progression of Alzheimer's disease are not well understood. Research indicates that the disease is associated with plaques and tangles in the brain. Current treatments only help with the symptoms of the disease. There are no available treatments that stop or reverse the progression of the disease. As of 2012[update], more than 1000 clinical trials have been or are being conducted to find ways to treat the disease, but it is unknown if any of the tested treatments will work. Mental stimulation, exercise, and a balanced diet have been suggested as possible ways to delay symptoms in healthy older individuals, but they have not been proven as effective. Because AD cannot be cured and is degenerative, the sufferer relies on others for assistance. The role of the main caregiver is often taken by the spouse or a close relative. Alzheimer's disease is known for placing a great burden on caregivers; the pressures can be wide-ranging, involving social, psychological, physical, and economic elements of the caregiver's life. In developed countries, AD is one of the most costly diseases to society.

Principle Of The Test

A monoclonal antibody specific for human AD7C-NTP has been coated onto the wells of the microtiter strips provided. Standards and Samples are pipetted into the wells and human AD7C-NTP binds to the immobilized antibody. After incubation, unbound substances is removed during a wash step, a detected antibody is added and binds to human AD7C-NTP captured by the first antibody. After incubation, unbound detected antibody is removed during a wash step. Streptavidin-HRP is added and binds to the detected antibody. After incubation, unbound streptavidin-HRP is removed during a wash step, and a chromogenic substrate solution is added to the wells and color develops in proportion to the amount of AD7C-NTP bound in the initial step. A colored product is formed and the reaction is terminated by the addition of stop solution. The intensity of the color is measured spectrophotometrically at 450 nm.

Reagents And Materials Provided

Human AD7C-NTP Ab Coated Wells, 96-well polystyrene microplate (12 strips of 8 wells): 1 plate
Human AD7C-NTP Standard(A-F, 0,0.25,1.0, 2.0, 5.0,10ng/mL): 0.8mL per vial with preservatives: 6 vials
Human AD7C-NTP Negative Control: 0.8mL per vial with preservatives: 1 vial
Human AD7C-NTP Positive Control: 0.8mL per vial with preservatives: 1 vial
Detected Antibody (Rabbit anti-Human AD7C-NTP PolyAntibody), 5 mL per vial with preservatives: 1 vial
Streptavidin-horseradish peroxidase (HRP) Concentrate, 5 mL per vial with preservatives: 2 vials
Wash Solution Concentrate (25×), 20 mL with preservatives: 1 bottle
Chromogen A, 5 mL per bottle: 1 bottle
Chromogen B, 5 mL per bottle: 1 bottle
Stop Solution, 5 mL per bottle: 1 bottle

Materials Required But Not Supplied

1. A standard ELISA plate reader for absorbance at 450 nm.
2. Calibrated adjustable precision pipettes (single channel and multi channel), preferably with disposable plastic tips.
3. Distilled or deionized water.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Data analysis and graphing software or graph paper.
6. Polypropylene tubes.
7. Graduated cylinders and calibrated beakers in various sizes.

Storage

Store all reagents at 2 to 8°C.

Specimen Collection And Handling

Urine: Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes and assay. Urine dilution is suggested at 1:2 into Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles

Reagent Preparation

Wash Solution: Make a 1:25 dilution of Wash Solution Concentrate (25X) with deionized or distilled water in a clean plastic tube as needed.

Assay Steps

Allow all reagents and samples to warm up to room temperature before use. It is recommended that all standards and samples be assayed in duplicate.
1. Determine the number of strips needed for the assay and remove excess microplate strips from the plate frame, return them to the foil pouch, and reseal.
2. Prepare standards and samples with appropriate diluents. Seal the plate with Plate Covers and incubate at 37°C for 60 min.
3. Add 100 μL of standards or dilutions of samples in duplicate to each well. Seal the plate with Plate Covers and incubate at 37°C for 60 min.
4. Aspirate each well and wash 5 times.
5. Add 100 μL detected antibody to each well except for the blank. Seal the plate with Plate Covers and incubate at 37°C for 30 min.
6. Aspirate each well and according to Step 4.
7. Add 100 μL of Streptavidin-HRP Solution to each well except for the blank. Seal the plate with Plate Covers and incubate at 37°C for 30 min.
8. Aspirate each well and wash according to Step 4.
9. Dispense 50μl of Chromogen A and 50μl Chromogen B solution into each well including the Blank and mix by tapping the plate gently. Incubate the plate at 37°C for 15 minutes avoiding light.
10. Add 50 μL of Stop Solution to each well.
11. Read the absorbance of each well at 450 nm within 10 minutes after adding the Stop Solution.
Quality Control

The OD value of the Positive control must be equal to or greater than 0.800 at 450/630nm or at 450nm after blanking.

Calculation

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density.

**Manual Plotting:** Plot on graph paper the absorbance of the standards against the standard concentration. Known concentrations of Human AD7C-NTP are plotted on the X-axis and the corresponding absorbances on the Y-axis. The standard curve should result in a straight line that shows a direct relationship between Human AD7C-NTP concentrations and the corresponding absorbances. The concentration of Human AD7C-NTP in samples may be determined by plotting the sample absorbances on the Y-axis, then drawing a horizontal line to intersect with the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding Human AD7C-NTP concentration.

Note: Samples producing signals greater than that of the highest standard should be diluted in Assay Solution and reanalyzed. Multiply the measured concentration by the appropriate dilution factor.

**Plate Reader:** An alternative approach is to use an ELISA curve fitting software. A linear curve plot is expected to produce the best fit of the resulting sample concentrations.

Sensitivity

The minimum detectable dose of human AD7C-NTP is < 0.25 ng/mL.

Specificity

No detectable cross-reactivity.

Linearity

Linear regression of samples versus the expected concentration yielded a correlation coefficient of 0.98.

Reproducibility

Intra-Assay: CV≤15%
Inter-Assay: CV≤20%

Precautions

1. This kit is for research use only, not for diagnostic or therapeutic procedures.
2. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells. Take care not to contaminate the Chromogen Solution. If the solution is blue before use, DO NOT USE.
3. This kit is intended ONLY for testing of individual serum samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
4. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible to avoid hemolysis of the RBC.