



User's Manual

Human anti- Acetylcholinesterase IgG ELISA Kit

REF

DEIASL418



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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

For the qualitative determination of human acetylcholinesterase(AChE) antibody(IgG) concentrations in serum.

General Description

Acetylcholinesterase, also known as AChE or acetylhydrolase, is a serine protease that hydrolyzes the neurotransmitter acetylcholine. AChE is found at mainly neuromuscular junctions and cholinergic brain synapses, where its activity serves to terminate synaptic transmission. It belongs to carboxylesterase family of enzymes.

Principles of Testing

This assay employs the qualitative enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with antigen. Samples are pipetted into the wells with anti-human IgG conjugated Horseradish Peroxidase (HRP). Any antibodies specific for the antigen present will bind to the pre-coated antigen. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of human acetylcholinesterase(AChE) antibody(IgG) bound in the initial step. The color development is stopped and the intensity of the color is measured.

Reagents And Materials Provided

1. Assay plate: 96 wells
2. Negative Control: 1 x 800 µl
3. Positive Control: 1 x 800 µl
4. Sample Diluent: 1 x 20 ml
5. HRP-conjugate: 1 x 10 ml
6. Wash Buffer (25 x concentrate): 1 x 20 ml
7. Substrate A: 1 x 5 ml
8. Substrate B: 1 x 5 ml
9. Stop Solution: 1 x 5 ml
10. Adhesive Strip (For 96 wells): 4
11. Instruction manual: 1

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 600 nm - 630 nm.
2. An incubator which can provide stable incubation conditions up to 37°C±0.5°C.

3. Squirt bottle, manifold dispenser, or automated microplate washer.
4. Absorbent paper for blotting the microtiter plate.
5. 100 mL and 500 mL graduated cylinders.
6. Deionized or distilled water.
7. Pipettes and pipette tips.
8. Test tubes for dilution.

Storage

The unopened kit should be stored at 2 - 8°C. Do not use the kit beyond the expiration date.

Opened kit may be stored for up to 1 month at 2 - 8°C.

Specimen Collection And Preparation

Serum: Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Sample Dilution

Serum samples require a 101-fold dilution into Sample Diluent before test. The suggested 101-fold dilution can be achieved by adding 2µl sample to 200µl of Sample Diluent.

Note:

1. Creative Diagnostics is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Samples to be used within 2 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1month) or -80°C (≤2month) to avoid loss of bioactivity and contamination.
3. Grossly hemolyzed samples are not suitable for use in this assay.
4. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
5. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Reagent Preparation

Note:

- (1) Kindly use graduated containers to prepare the reagent.
- (2) Bring all reagents to room temperature (18-25°C) before use for 30min.
- (3) Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

Wash Buffer(1x)- If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate (25 x) into deionized or

distilled water to prepare 500 ml of Wash Buffer (1 x).

Assay Procedure

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, and samples as directed in the previous sections.
2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Set a Blank well without any solution.
4. Add 100µl of Negative Control, Positive Control or diluted Sample per well. Cover with the adhesive strip provided. Incubate for 30 minutes at 37°C.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (300µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100µl of HRP-conjugate to each well (not to Blank!). Cover the microtiter plate with the adhesive strip. Incubate for 30 minutes at 37°C.
7. Repeat the aspiration/wash process for five times as in step 5.
8. Add 50µl of Substrate A and 50µl Substrate B to each well. Incubate for 10 minutes at 37°C. Protect from light.
9. Add 50µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
10. Take blank well as zero, determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

Calculation

For calculation the valence of human acetylcholinesterase(AChE) antibody(IgG), compare the sample well with control.

The negative control OD value should be less than 0.1, and the positive control OD value should be greater than 0.800. Or else, repeat the test.

If $OD_{negative} < 0.1$, calculate it as 0.1.

While $OD_{sample} / OD_{negative} \geq 2.1$: Positive

While $OD_{sample} / OD_{negative} < 2.1$: Negative

Precision

Intra-assay Precision (Precision within an assay): $CV\% < 15\%$

Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): $CV\% < 15\%$

Three samples of known concentration were tested in twenty assays to assess.

Specificity

This assay has high sensitivity and excellent specificity for detection of human acetylcholinesterase(AChE) antibody(IgG). No significant cross-reactivity or interference between human acetylcholinesterase(AChE) antibody(IgG) and analogues was observed. Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between human acetylcholinesterase(AChE) antibody(IgG) and all the analogues, therefore, cross reaction may still exist.

Precautions

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Limitations

1. FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
2. The kit should not be used beyond the expiration date on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.
4. Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
5. This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.