



## User's Manual

# Rat Acetylcholinesterase ELISA Kit

REF

DEIASL417



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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.

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### Creative Diagnostics

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## PRODUCT INFORMATION

### Intended Use

For the quantitative determination of rat acetylcholinesterase (AChE) concentrations in serum, plasma, tissue homogenates.

### 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 quantitative sandwich enzyme immunoassay technique. Antibody specific for AChE has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any AChE present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for AChE is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of AChE 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 (12 x 8 coated Microwells): 1(96 wells)
2. Standard (Freeze dried): 2
3. Biotin-antibody (100 x concentrate): 1 x 120 µl
4. HRP-avidin (100 x concentrate): 1 x 120 µl
5. Biotin-antibody Diluent: 1 x 15 ml
6. HRP-avidin Diluent: 1 x 15 ml
7. Sample Diluent: 1 x 50 ml
8. Wash Buffer (25 x concentrate): 1 x 20 ml
9. TMB Substrate: 1 x 10 ml
10. Stop Solution: 1 x 10 ml
11. Adhesive Strip (For 96 wells): 4
12. 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  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ .
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  $2 - 8^{\circ}\text{C}$ . Do not use the kit beyond the expiration date.

Opened kit may be stored for up to 1 month at  $2 - 8^{\circ}\text{C}$ .

## 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  $4^{\circ}\text{C}$  before centrifugation for 15 minutes at 1000 xg. Remove serum and assay immediately or aliquot and store samples at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**Plasma:** Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 xg at  $2-8^{\circ}\text{C}$  within 30 minutes of collection. Assay immediately or aliquot and store samples at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**Tissue Homogenates:** 100mg tissue was rinsed with 1X PBS, homogenized in 1 ml of 1X PBS and stored overnight at  $-20^{\circ}\text{C}$ . After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g,  $2 - 8^{\circ}\text{C}$ . The supernate was removed and assayed immediately. Alternatively, aliquot and store samples at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$ . Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Recommend to dilute the serum or plasma samples with Sample Diluent (1:200) before test. The suggested 200-fold dilution can be achieved by adding 5 $\mu\text{l}$  sample to 45 $\mu\text{l}$  of Sample Diluent first, then complete the 200-fold dilution by adding 15 $\mu\text{l}$  of this solution to 285 $\mu\text{l}$  of Sample Diluent. The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments.

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 5 days may be stored at  $2-8^{\circ}\text{C}$ , otherwise samples must be stored at  $-20^{\circ}\text{C}$  ( $\leq 1\text{month}$ ) or  $-80^{\circ}\text{C}$  ( $\leq 2\text{month}$ ) 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. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
6. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
7. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
8. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
9. 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. Please don't prepare the reagent directly in the Diluent vials provided in the kit.

(2) Bring all reagents to room temperature (18-25°C) before use for 30min.

(3) Prepare fresh standard for each assay. Use within 4 hours and discard after use.

(4) Making serial dilution in the wells directly is not permitted.

(5) Please carefully reconstitute Standards according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µl for once pipetting.

(6) 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.

1. Biotin-antibody (1x) - Centrifuge the vial before opening.

Biotin-antibody requires a 100-fold dilution. A suggested 100-fold dilution is 10 µl of Biotin-antibody + 990 µl of Biotin-antibody Diluent.

2. HRP-avidin (1x) - Centrifuge the vial before opening.

HRP-avidin requires a 100-fold dilution. A suggested 100-fold dilution is 10 µl of HRP-avidin + 990 µl of HRP-avidin Diluent.

3. 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).

4. Standard

Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the Standard with 1.0 ml of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution of 500 pg/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µl of Sample Diluent into each tube (S0-S6). Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (500 pg/ml). Sample Diluent serves as the zero standard

(0 pg/ml).

Tube	S7	S6	S5	S4	S3	S2	S1	S0
pg/ml	500	250	125	62.5	31.2	15.6	7.8	0

## 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, working standards, 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. Add 100µl of standard and sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at 37°C. A plate layout is provided to record standards and samples assayed.
4. Remove the liquid of each well, don't wash.
5. Add 100µl of Biotin-antibody (1x) to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37°C. (Biotin-antibody (1x) may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.)
6. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (200µl) 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.
7. Add 100µl of HRP-avidin (1x) to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 1 hour at 37°C.
8. Repeat the aspiration/wash process for five times as in step 6.
9. Add 90µl of TMB Substrate to each well. Incubate for 15-30 minutes at 37°C. Protect from light.
10. Add 50µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

## Calculation

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

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the AChE concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## Typical Standard Curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

pg/ml	OD1	OD2	Average	Corrected
500	2.361	2.384	2.373	2.232
250	1.636	1.658	1.647	1.506
125	1.044	1.057	1.051	0.910
62.5	0.610	0.599	0.605	0.464
31.2	0.413	0.404	0.409	0.268
15.6	0.305	0.297	0.301	0.160
7.8	0.237	0.228	0.233	0.092
0	0.142	0.140	0.141	

## Precision

Intra-assay Precision (Precision within an assay): CV%<8%

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

Inter-assay Precision (Precision between assays): CV%<10%

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

## Detection Range

7.8 pg/ml-500 pg/ml.

## Sensitivity

The minimum detectable dose of rat AChE is typically less than 1.95 pg/ml. The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined the mean O.D value of 20 replicates of the zero standard added by their three standard deviations.

## Specificity

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

## Linearity

To assess the linearity of the assay, samples were spiked with high concentrations of rat AChE in various matrices and diluted with the Sample Diluent to produce samples with values within the dynamic range of the assay.

	Sample	Serum(n=4)
1:100	Average %	92
	Range %	88-95
1:200	Average %	96
	Range %	90-101
1:400	Average %	94
	Range %	90-97
1:800	Average %	99
	Range %	95-103

## Recovery

The recovery of rat AChE spiked to levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay as directed in the Sample Preparation section.

Sample Type	Average % Recovery	Range
Serum (n=5)	95	91-99
EDTA plasma (n=4)	92	87-96

## 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. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
5. Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
6. 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.

