XpressBio

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Mouse AGER (Advanced Glycosylation End Product Specific Receptor) ELISA Kit

Catalogue No.: XPEM0526

Size: 48T/96T

Reactivity: Mouse

Range: 125-8000pg/ml

Sensitivity: <75pg/ml

Application: For quantitative detection of AGER in serum, plasma, tissue homogenates and other biological fluids.

Storage: 4°C for 6 months

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications(48T/96T)	Storage
Micro ELISA Plate(Dismountable)	8×6 or 8×12	4°C/-20°C
Lyophilized Standard	1 vial or 2 vial	4°C/-20°C
Sample / Standard dilution buffer	10ml/20ml	4°C
Biotin-detection antibody (Concentrated)	60ul/120ul	4°C
Antibody dilution buffer	5ml/10ml	4°C
HRP-Streptavidin Conjugate(SABC)	60ul/120ul	4°C(shading light)
SABC dilution buffer	5ml/10ml	4°C
TMB substrate	5ml/10ml	4°C(shading light)
Stop solution	5ml/10ml	4°C
Wash buffer (25X)	15ml/30ml	4°C
Plate Sealer	3/5pieces	
Product Description	1 copy	

Principle of the Assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti-AGER antibody was pre-coated onto 96-well plates. And the biotin conjugated anti-AGER antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection

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antibody were added to the wells subsequently, and wash with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the AGER amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of AGER can be calculated.

Precautions for Use

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don’t let Micro plate dry at the assay, for dry plate will inactivate active components on plate.
8. Don’t reuse tips and tubes to avoid cross contamination.
9. Avoid using the reagents from different batches together.

Material Required but Not Supplied

1. Microplate reader (wavelength: 450nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Manual Washing

Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a total of THREE washes.

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Automated Washing

Aspirate all wells, then wash plate THREE times with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute.

Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

- Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.
- Plasma: Collect plasma using EDTA-Na2 as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
- Tissue homogenates: For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000×g to get the supernatant.
- Cell culture supernatant: Centrifuge supernatant for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8°C. Collect the clear supernatant and carry out the assay immediately.
- Other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2 - 8°C. Collect the supernatant and carry out the assay immediately.
- Sample preparation: Samples should be clear and transparent and be centrifuged to remove suspended solids.

Note: Samples to be used within 5 days may be stored at 4°C,otherwise samples must be stored at -20°C (≤1 month) or -80°C(≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

Sample Dilution Guideline

End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration falls the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary in practice. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be make in pre-experiment.

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- High target protein concentration (80000-800000pg/ml): Dilution: 1:100. (i.e. Add 1µl of sample into 99 µl of Sample / Standard dilution buffer.)
- Medium target protein concentration (8000-80000pg/ml): Dilution: 1:10.(i.e. Add 10 µl of sample into 90 µl of Sample / Standard dilution buffer.)
- Low target protein concentration (125-8000pg/ml): Dilution: 1:2.(i.e. Add 50 µl of sample into 50 µl of Sample / Standard dilution buffer.)
- Very low target protein concentration (≤125pg/ml): Unnecessary to dilute, or dilute at 1:2.

Reagent Preparation and Storage

Bring all reagents to room temperature before use.

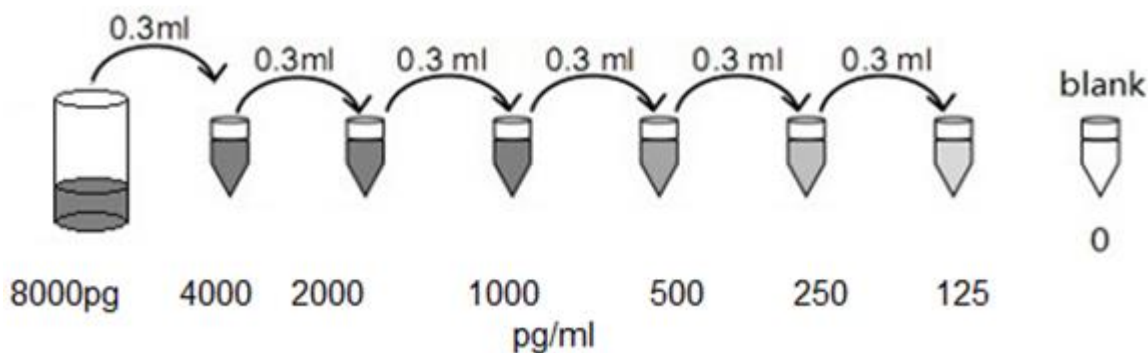
1, Wash Buffer:

Dilute 30mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

2, Standard:

1). 8000pg/ml of standard solution: Add 1 ml of Sample / Standard dilution buffer into one Standard tube, keep the tube at room temperature for 10 min and mix thoroughly.

2). 4000pg/ml→125pg/ml of standard solutions: Label 6 Eppendorf tubes with 4000pg/ml, 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, respectively. Aliquot 0.3 ml of the Sample / Standard dilution buffer into each tube. Add 0.3 ml of the above 8000pg/ml standard solution into 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.



Note: The standard solutions are best used within 2 hours. The standard solution should be at 4°C for up to 12 hours. Or store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

3, Preparation of Biotin-detection Antibody working solution

Prepare within 1 hour before the experiment.

1) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

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2) Dilute the Biotin-detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly.(i.e. Add 1 µl of Biotin-detection antibody into 99 µl of Antibody dilution buffer.)

4, Preparation of HRP-Streptavidin Conjugate (SABC) working solution:

Prepare within 30 minutes before the experiment.

1) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)
2) Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1 µl of SABC into 99 µl of SABC dilution buffer.)

Assay Procedure

Before adding to wells, equilibrate the SABC working solution and TMB substrate for at least 30 min at 37 °C. When diluting samples and reagents, they must be mixed completely and evenly. It is recommend to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommend to measure each standard and sample in duplicate. **Wash plate 2 times before adding standard, sample and control (zero) wells!**
2. Aliquot 0.1ml of 8000pg/ml, 4000pg/ml, 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, standard solutions into the standard wells.
3. Add 0.1 ml of Sample / Standard dilution buffer into the control (zero) well.
4. Add 0.1 ml of properly diluted sample (Mouse serum, plasma, tissue homogenates and other biological fluids.) into test sample wells.
5. Seal the plate with a cover and incubate at 37 °C for 90 min.
6. Remove the cover and discard the plate content, and wash plate 2 times with Wash Buffer. Do NOT let the wells dry completely at any time.
7. Add 0.1 ml of Biotin-detection antibody working solution into the above wells (standard, test sample & zero wells). Add the solution at the bottom of each well without touching the side wall.
8. Seal the plate with a cover and incubate at 37°C for 60 min.
9. Remove the cover, and wash plate 3 times with Wash buffer.
10. Add 0.1 ml of SABC working solution into each well, cover the plate and incubate at 37°C for 30 min.
11. Remove the cover and wash plate 5 times with Wash buffer, and each time let the wash buffer stay in the wells for 1-2 min.
12. Add 90 µl of TMB substrate into each well, cover the plate and incubate at 37°C in dark within 15-30 min. (Note: This incubation time is for reference use only, the optimal time should be determined by end user.) And the shades of blue can be seen in the first 3-4 wells (with most concentrated AGER standard solutions), the other wells show no obvious color.

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13. Add 50 µl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.
14. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The AGER concentration of the samples can be interpolated from the standard curve. Recommended to use professional software curve expert to 1.3,for details, please visit: www.xpressbio.com

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Summary

1. Wash plate 2 times before adding standard, sample and control (zero) wells!
2. Add 100µL standard or sample to each well for 90 minutes at 37°C
3. Add 100µL Biotin-detection antibody working solution to each well for 60 minutes at 37°C
4. Aspirate and wash 3 times
5. Add 100µL SABC working solution to each well. Incubate for 30 minutes at 37°C
6. Aspirate and wash 5 times
7. Add 90µLTMB substrate. Incubate 15 -30 minutes at 37°C
8. Add 50µL Stop Solution. Read at 450nm immediately
9. Calculation of results

Typical Data & Standard Curve

Results of a typical standard run of a AGER ELISA Kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment. (N/A=not applicable)

X	pg/ml	0	125	250	500	1000	2000	4000	8000
Y	OD450	0.015	0.052	0.097	0.179	0.351	0.719	1.528	2.349

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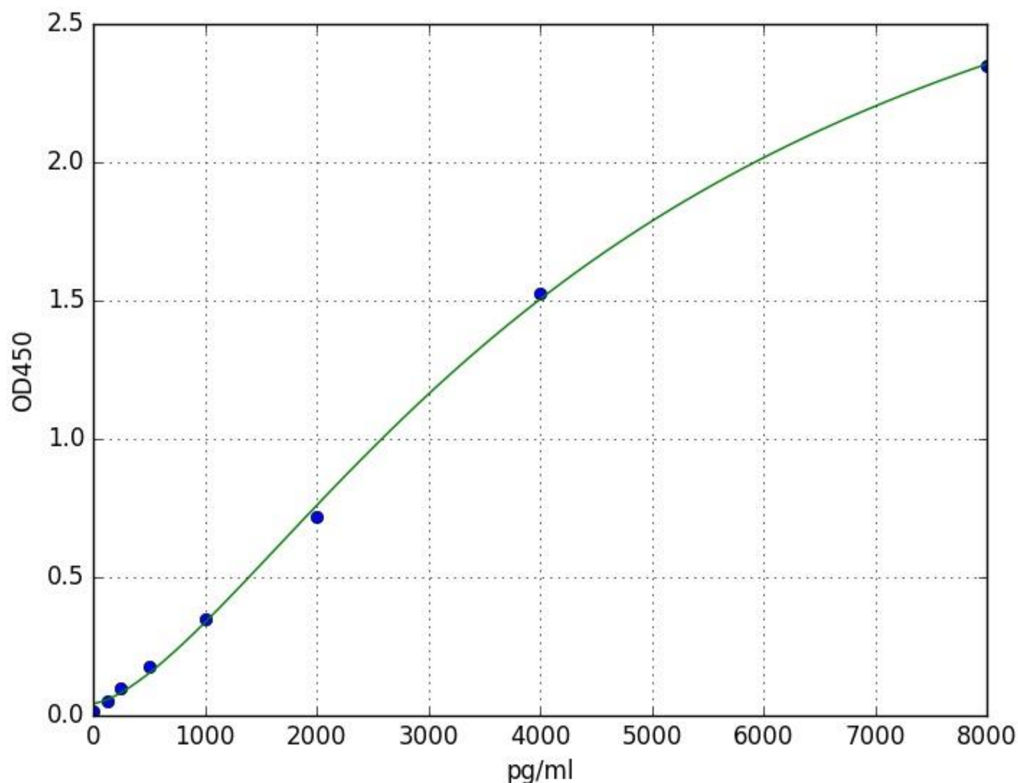

Specificity

This assay has high sensitivity and excellent specificity for detection of AGER . No significant cross-reactivity or interference between AGER and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between AGER and all the analogues, therefore, cross reaction may still exist.

Recovery

Matrices listed below were spiked with certain level of AGER and the recovery rates were calculated by comparing the measured value to the expected amount of AGER in samples.

Matrix	Recovery range (%)	Average(%)
serum(n=5)	86-103	94
EDTA plasma(n=5)	85-102	93
heparin plasma(n=5)	89-101	94

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Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of AGER and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8	1:16
serum(n=5)	85-97%	90-105%	88-104%	87-105%
EDTA plasma(n=5)	83-100%	85-101%	88-101%	82-98%
heparin plasma(n=5)	80-98%	84-100%	88-97%	82-95%

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level AGER were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level AGER were tested on 3 different plates, 8 replicates in each plate.

CV(%) = SD/meanX100

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage condition.

Standard(n=5)	37°C for 1 month	4°C for 6 months
Average(%)	80	95-100

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.