Product Manual

Revision date: 05 Nov 2019

Human Advanced Glycosylation End Product Specific Receptor (AGER) CLIA Kit

Catalog No.: abx196212

Size: 96T

Range: 15.6 pg/ml - 1000 pg/ml

Sensitivity: 9.38 pg/ml

Storage: Store the 96-well plate, Standards, HRP-conjugate reagent and Biotin-conjugated antibody at -20°C, and the rest of the kit components at 4°C.

Application: For quantitative detection of AGER in Human Serum, Plasma and other biological fluids.

Introduction: RAGE (receptor for advanced glycation endproducts), also called AGER, is a 35 kilodalton transmembrane receptor of the immunoglobulin super family which was first characterized in 1992 by Neeper et al. Its name comes from its ability to bind advanced glycation endproducts (AGE), which include chiefly glycoproteins, the glycans of which have been modified non-enzymatically through the Maillard reaction. In view of its inflammatory function in innate immunity and its ability to detect a class of ligands through a common structural motif, RAGE is often referred to as a pattern recognition receptor. RAGE also has at least one other agonistic ligand: high mobility group protein B1 (HMGB1). HMGB1 is an intracellular DNA-binding protein important in chromatin remodeling which can be released by necrotic cells passively, and by active secretion from macrophages, natural killer cells, and dendritic cells.

Principle of the Assay

This kit is based on sandwich chemiluminescent immunoassay technology. Anti-AGER antibody is pre-coated onto a 96-well plate. The standards and samples are added to the wells and incubated. Biotin-conjugated anti-AGER and avidin conjugated to HRP are added to each microplate well and incubated. After the mixture of substrate A and B is added only wells that contain AGER will produce chemiluminescence. The intensity of the emitted light is proportional to the amount of AGER in the sample or standard.

Kit components

1. One pre-coated 96-well microplate (12 × 8 well strips)
2. Standard: 2 tubes
3. Sample/Standard Diluent Buffer: 20 ml
4. Biotin conjugated antibody (Dilution 1:100): 120 µl
5. Antibody diluent buffer: 12 ml
6. HRP Conjugate Reagent (Dilution 1:100): 120 µl
7. HRP diluent buffer: 12 ml
8. Substrate Reagent A: 5 ml
9. Substrate Reagent B: 5 ml
10. Wash buffer (25X): 30 ml
11. Plate Sealer: 5

Material Required But Not Provided

1. 37°C incubator
2. Luminometer capable of reading 96-well microplates
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. 1.5 ml tubes to prepare standard/sample dilutions
6. Absorbent filter papers
7. 100 ml and 1 liter graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.

» Samples should be clear and transparent. Samples must be diluted so that the expected concentration falls within the kit's range.

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used. Samples that contain NaN_3 cannot be detected as it interferes with HRP.

» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

2. Wash buffer

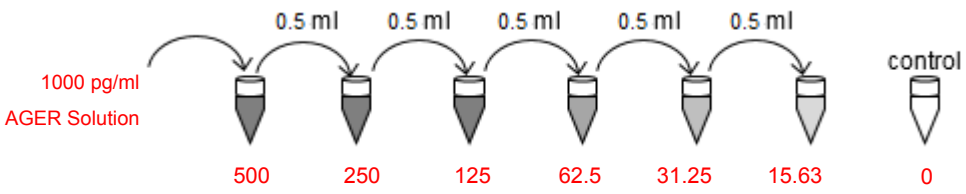
Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water).

3. Standard

Preparation of the AGER standard: standard solution should be prepared no more than 15 min prior to the experiment. Centrifuge at 10,000×g for 1 minute.

a.) 1000 pg/ml standard solution. Add 1 ml of Sample/Standard diluent buffer into one Standard tube. Allow the reconstituted standard to sit for 15 minutes with gentle agitation prior to carrying out the serial dilutions; avoiding foaming or bubbles.

b.) 500 pg/ml → 15.625 pg/ml standard solutions: Label 6 tubes with 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml and 15.625 pg/ml. Aliquot 0.5 ml of the Sample / Standard diluent buffer into each tube. Add 0.5 ml of the above 1000 pg/ml standard solution into 1st tube and mix thoroughly. Transfer 0.5 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.5 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.



Note: Do not vortex the standard during reconstitution, as this will destabilize the protein. Once your standard has been reconstituted, it should be used right away. Use the diluted Standards for a single assay procedure and discard after use.

4. Preparation of Biotin conjugated antibody working solution: prepare no more than 1 hour before the experiment.

a.) 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).

b.) Dilute the Biotin conjugated antibody with antibody diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of Biotin conjugated antibody into 99 µl of antibody diluent buffer. Discard after use.

5. Preparation of HRP Conjugated Reagent working solution: prepare no more than 30 min. before the experiment
a.) 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).
b.) Dilute the HRP Conjugate Reagent with HRP diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of HRP Conjugate Reagent into 99 µl of HRP diluent buffer. Discard after use.

5. Preparation of Substrate mixture solution:
a.) Calculate the total volume of the mixture solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) In a separate tube, add Substrate Reagent A and Substrate Reagent B in a 1:1 ratio and mix thoroughly. Discard after use.

B. Assay Procedure

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate. Mix the standards and samples up and down to be homogeneous before adding into the wells but avoid adding bubbles.
2. Add 100 µl of the prepared standards solutions into the standard wells.
3. Add 100 µl of Sample / Standard diluent buffer into the control (zero) well.
4. Add 100 µl of appropriately diluted sample into test sample wells.
5. Cover the plate and incubate at 37°C for 90 minutes.
6. Remove the cover and discard the liquid. Do not wash.
7. Add 100 µl of prepared Biotin conjugated antibody working solution into each well (standard, test sample and zero well). Add the solution at the bottom of each well without touching the side walls. Seal the plate with a cover and incubate at 37°C for 60 minutes.
8. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer. Fill each well completely with Wash buffer (350 µl) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
9. Add 100 µl of HRP working solution into each well, cover the plate and incubate at 37°C for 30 minutes.
10. Remove the cover, discard the liquid and wash the plate 5 times with Wash Buffer as explained in step 8.
11. Add 100 µl of Substrate mixture solution into each well. Cover the plate and incubate at 37°C in dark conditions for up to 5 minutes.
12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the chemiluminescence signal in a microplate luminometer immediately.

For calculation, average the duplicate readings for each set of reference standard, control and samples and substract the average zero standard RLU (Relative Light Unit). The standard curve can be plotted with the mean RLU of each reference standard on the Y axis vs. the respective concentration of each standard solution on the X axis. The AGER concentration of the samples can be interpolated from the standard curve.

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

C. Precautions

1. Equilibrate all reagents to room temperature (18-25°C) prior to use and centrifuge the tubes briefly in case any contents are trapped in the lid.
2. If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. Avoid foaming or bubbles when mixing or reconstituting components. Prepare the Standard dilutions within 15 min of use and discard any unused working standards. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
4. It is recommended measuring each standard and sample in duplicate.
5. Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells, avoid letting the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
6. Ensure plates are properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
8. Do not reuse pipette tips and tubes to avoid cross contamination.
9. The Substrate solution is easily contaminated; work under sterile conditions when handling the substrate solution. The Substrate A and B should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Precision

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

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

CV (%) = (Standard Deviation / mean) × 100

Intra-Assay: CV<10%

Inter-Assay: CV<10%