Version 05-05/24

User's Manual

Mouse Angiotensinogen ELISA Kit

REF **DEIA-XY108NS**

Σ **96T**

RUO

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

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of AGT in mouse serum, plasma, tissue homogenates and other biological fluids.

Principles of Testing

The microtiter plate provided in this kit has been pre-coated with an antibody specific to AGT. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody preparation specific to AGT. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain AGT, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 ± 10 nm. The concentration of AGT in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Reagents And Materials Provided

1. Pre-coated, ready to use 96-well strip plate, 1

2. Standard, 2

3. Detection Solution A, 1 × 12 mL

4. Detection Solution B, 1 × 12 mL

5. Wash Buffer (30× concentrate), 1 × 20 mL

6. Plate sealer for 96 wells, 2

7. Standard Diluent, 1 × 20 mL

8. TMB Substrate, 1 × 9 mL

9. Stop Solution, 1 × 6 mL

10. Instruction manual, 1

Materials Required But Not Supplied

1. Microplate reader with 450 ± 10 nm filter.
2. Single or multi-channel pipettes and disposable tips.
3. Eppendorf Tubes for diluting samples.
4. Deionized or distilled water.
5. Absorbent paper for blotting the microtiter plate.
6. Container for Wash Solution.

Storage

Store all components at 4°C

Specimen Collection And Preparation

1. Serum: Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000 × g. Assay freshly prepared serum immediately or store samples in aliquots at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

2. Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 × g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquots at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

3. Tissue homogenates: The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissues should be rinsed in ice-cold PBS (0.01 mol/L, pH 7.0-7.2) to remove excess blood thoroughly and weighed before homogenization. Mince the tissues to small pieces and homogenize them in 5-10 mL of PBS with a glass homogenizer on ice (Micro Tissue Grinders also work). The resulting suspension should be sonicated with an ultrasonic cell disrupter or subjected to 2 freeze/thaw cycles to further break the cell membranes. Then, centrifuge the homogenates for 5 minutes at 5000 × g. Remove the supernatant and assay immediately or aliquot and store at ≤-20°C.

4. Other biological fluids: Centrifuge samples for 20 minutes at 1000 × g. Remove particulates and assay immediately or store samples in aliquots at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Note:

1. 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/or contamination.
2. Noticeable hemolysis will affect antibody-antigen reactions. Samples with any sign of hemolysis are not acceptable for this assay.
3. When performing the assay, bring samples to room temperature.

SAMPLE PREPERATION

1. CD is only responsible for the kit itself, not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole assay. Please reserve sufficient samples in advance.
2. 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 specific experiments. Samples should be diluted by 0.01 mol/L PBS (pH 7.0-7.2).
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. Tissue or cell extraction samples prepared using a chemical lysis buffer may cause unexpected ELISA results due to the impacts from certain chemicals.
5. Due to the possibility of mismatching between antigens from other origin and antibodies 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.

6. Samples from cell culture supernatant may not be detected by the kit due to influence from factors such as cell viability, cell number and/or sampling time.
7. Fresh samples are recommended for the assay. Protein degradation and denaturation may occur in samples stored over extensive periods of time and may lead to inaccurate or incorrect results.

Reagent Preparation

1. Bring all kit components and samples to room temperature (18-25°C) before use.
2. **Standard:** Reconstitute the Standard with 1.0 mL of Standard Diluent, keep for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 40 ng/mL. Prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Prepare a dilution series with 7 points; for example: 40 ng/mL, 20 ng/mL, 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL, and the last EP tube with Standard Diluent is the blank at 0 ng/mL.
3. **Detection Solution A and Detection Solution B:** Detection Solutions A and B are already at the correct concentrations and do not need to be diluted further.
4. **Wash Solution:** Dilute 20 mL of Wash Solution concentrate (30×) with 580 mL of deionized or distilled water to prepare 600 mL of Wash Solution (1×).
5. **TMB substrate:** Aspirate the needed dosage of the solution with sterilized tips. Do not dump the residual solution back into the vial.

Note:

1. Do not perform a serial dilution directly in the wells.
2. Prepare standard within 15 minutes of performing the assay.
3. Carefully reconstitute Standards according to the instruction, avoid foaming and mix gently until the crystals are completely dissolved.
4. The reconstituted Standards can be used only once.
5. If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gently until the crystals are completely dissolved.
6. Any contaminated water or container used during reagent preparation will influence the detection result.

Assay Procedure

1. Determine wells for diluted standard, blank and sample. Prepare 7 wells for the standards, 1 well for blank. Add 100 µL each of dilutions of standard (read Reagent Preparation), blank, and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 90 minutes at 37°C.
2. Remove the liquid from each well, do not wash.
3. Add 100 µL of Detection Solution A to each well. Incubate for 45 minutes at 37°C after covering it with the Plate sealer.
4. Aspirate the solution and wash with 300 µL of 1× Wash Solution to each well using a squirt bottle, multichannel pipette, manifold dispenser or auto-washer, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by tapping the plate onto absorbent paper. Wash thoroughly 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate

and blot it against absorbent paper.

5. Add 100 µL of Detection Solution B to each well. Incubate for 45 minutes at 37°C after covering it with the Plate sealer.
6. Repeat the aspiration/wash process for a total of 5 times as conducted in step 4.
7. Add 90 µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 15-25 minutes at 37°C (Do not exceed 30 minutes). Protect from light. The liquid will turn blue with the addition of the Substrate Solution.
8. Add 50 µL of Stop Solution to each well. The liquid will turn yellow with the addition of the Stop solution. Mix the liquid by tapping the side of the plate. If the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Remove any drops of water and fingerprints on the bottom of the plate and confirm there are no bubbles on the surface of the liquid. Run the microplate reader and take measurements at 450 nm immediately.

Note:

1. Assay preparation: Keep appropriate numbers of wells for each experiment and remove extra wells from microplate. Remaining wells should be resealed and stored at -20°C.
2. Samples or reagents addition: Please use the freshly prepared Standard. Carefully add samples to wells and mix gently to avoid foaming. Do not touch the well walls. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of standards, samples, and reagents. In addition, use separated reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents are added to the well strips, DO NOT let the strips dry at any time during the assay. Incubation time and temperature must be controlled.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting, and remove any drops of water or fingerprints on the bottom of the plate. Insufficient washing will result in poor precision and false elevated absorbance reading.
5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g., observation once every 10 minutes), if the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in an inaccurate absorbance reading.
6. TMB Substrate is light sensitive and easily contaminated by oxidizing agents. Please protect it from light exposure and potential sources of contamination.
7. The environment humidity may influence the results obtained from the kit. If the humidity in your facility is less than 60%, using a humidifier is recommended.

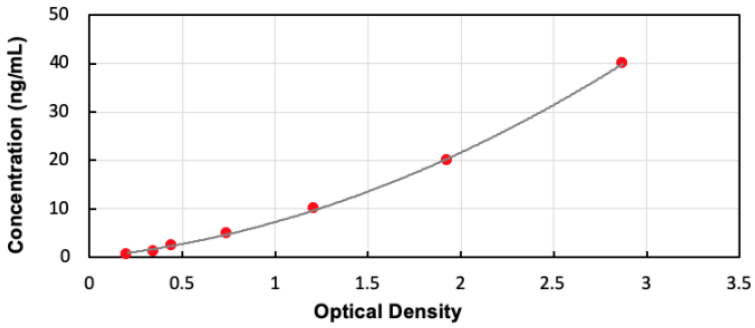
Calculation

Average the duplicate readings for each standard, control and sample, then subtract the average zero standard optical density. Construct a standard curve by plotting the mean O.D. and concentration for each standard and draw a best fit curve through the points on the graph or create a standard curve on log-log

graph paper with AGT concentration on the y-axis and absorbance on the x-axis. Using plotting software, (for instance, curve expert 1.30), is also recommended. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Standard Curve

To make the calculation easier, we plot the O.D. value of the standard (x-axis) against the known concentration of the standard (y-axis), although concentration is the independent variable and O.D. value is the dependent variable. However, the O.D. values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique or temperature effects), plotting the log of the data to establish a standard curve for each test is recommended. The typical standard curve below is provided for reference only.



Precision

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

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

CV (%) = SD/mean × 100

Intra-Assay: CV<10%

Inter-Assay: CV<12%

Detection Range

0.625-40 ng/mL. The concentrations used for creating the standard curve were 40 ng/mL, 20 ng/mL, 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL.

Sensitivity

The minimum detectable dose of AGT is typically less than 0.226 ng/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 by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding

concentration.

Specificity

This assay has high sensitivity and excellent specificity for detection of AGT.

No significant cross-reactivity or interference between AGT and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible to perform all possible cross-reactivity detection tests between AGT and all analogues, therefore, cross reactivity may still exist.

Limitations

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

This material is sold for in-vitro use only in manufacturing and research. This material is not suitable for human use. It is the responsibility of the user to undertake sufficient verification and testing to determine the suitability of each product's application. The statements herein are offered for informational purposes only and are intended to be used solely for your consideration, investigation and verification