Monkey Albumin (ALB) ELISA Kit

Catalog Number: EK20494

The kit is a competitive inhibition enzyme immunoassay technique for the in vitro quantitative measurement of ALB in monkey serum, plasma or other biological fluids.

This instruction must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

Contact information

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REAGENTS AND MATERIALS PROVIDED

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	2
Standard	2	Standard Diluent	1×20mL
Detection Reagent B	1×60μL	Assay Diluent B	1×12mL
TMB Substrate	1×9mL	Stop Solution	1×6mL
Wash Buffer (25 × concentrate)	1×20mL	Instruction manual	1

MATERIALS REQUIRED BUT NOT SUPPLIED

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

STORAGE OF THE KITS

1. For unopened kit: All the reagents should be kept accordiµg to the labels on vials. The **TMB Substrate, Wash Buffer (25 × concentrate)** and the **Stop Solution** should be stored at **4ºC** upon receipt while **the others** should be at **-20ºC**.
2. For opened kits: Once the kit is opened, the remainiµg reagents still need to be stored accordiµg to the above storage conditions. In addition, return the unused wells to the foil pouch containiµg the desiccant pack and reseal aloµg entire edge of zip-seal.

Note:

For the expiration date of the kit, please refer to the label on the kit box. All components are stable until this expiration date.

It is highly recommended to use the remainiµg reagents within 1 month of openiµg.

SAMPLE COLLECTION AND STORAGE

- **Serum** - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4ºC 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.

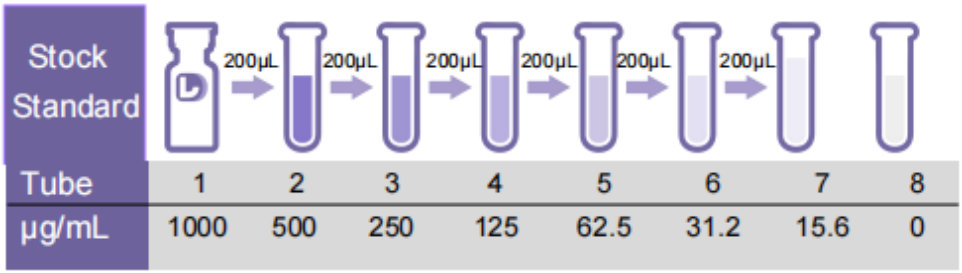
- **Plasma** - Collect plasma usiµg EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquots at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.
- **Other 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 contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen can not be detected.
3. When performiµg the assay, briµg samples to room temperature.

REAGENT PREPARATION

1. **Briμg all kit** components and samples to room temperature (18-25°C) before use.
2. **Standard** - Reconstitute the Standard with 1.0mL of Diluent Buffer, keep for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 1000μg/mL. Prepare 7 tubes containiμg 0.2mL Diluent Buffer and use the diluted standard to produce a double dilution series accordiμg to the picture shown below. Mix each tube thoroughly before the next transfer. Prepare a dilution series with 7 points; for example: 1000μg/mL, 500μg/mL, 250μg/mL, 125μg/mL, 62.5μg/mL, 31.2μg/mL, 15.6μg/mL, and the last EP tube with Diluent Buffer is the blank at 0μg/mL.



Tube	1	2	3	4	5	6	7	8
μg/mL	1000	500	250	125	62.5	31.2	15.6	0

3. **Detection Reagent B** - Briefly spin or centrifuge the stock Detection B before use. Dilute to the workiμg concentration with workiμg Assay Diluent B, respectively (1:100).
4. **Wash Solution** - Dilute 20mL of Wash Solution concentrate (25×) with 480mL of deionized or distilled water to prepare 500 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 before assay. Do not dissolve the reagents at 37°C directly.
3. Detection Reagent B are sticky solutions, therefore slowly pipette them to reduce the volume errors.
4. Carefully reconstitute Standards or workiμg Detection Reagent B accordiμg to the instruction, and avoid foamiμg and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipettiμg, use small volumes and ensure that pipettors are calibrated. It is recommended to pipette more than 10μL at a time to ensure accuracy.

5. The reconstituted Standards, Detection Reagent B can be **used only once**.
6. If crystals have formed in the Wash Solution concentrate (25×), warm to room temperature and mix gently until the crystals are completely dissolved.
7. Any contaminated water or container used duriµg reagent preparation will influence the detection result.

SAMPLE PREPARATION

1. SAB is only responsible for the kit itself, but not for the samples consumed duriµg the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Please predict the concentration before assayiµg. If values for these are not within the raµge of the standard curve, users must determine the optimal sample dilutions for their particular experiments. Samples should be diluted by 0.01mol/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 usiµg a chemical lysis buffer may cause unexpected ELISA results due to the impacts

from certain chemicals.

5. Due to the possibility of mismatchiµg 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 sampliµg time.
7. Fresh samples that have not been stored for extended periods of time are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and give inaccurate or incorrect results.

ASSAY PROCEDURE

1. Determine wells for diluted standard, blank and sample. Prepare 7 wells for standard, 1 well for blank. Add 50µL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells, respectively. And then add 50µL of Detection Reagent B to each well immediately. Shake the plate gently (usiµg a microplate shaker is recommended). Cover with a Plate sealer. Incubate for 1 hour at 37ºC.

Detection Reagent B may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.

2. Aspirate the solution and wash with 300μL of 1× Wash Solution to each well usiμg a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remainiμg liquid from all wells completely by tappiμg the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remainiμg Wash Buffer by aspiratiμg or decantiμg. Invert the plate and blot it against absorbent paper.
3. Add 90μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 15-25 minutes at 37ºC (Do not exceed 30 minutes). Protect from light. The liquid will turn blue with the addition of Substrate Solution.
4. Add 50μL of Stop Solution to each well. The liquid will turn yellow with the addition of Stop solution. Mix the liquid by tappiμg the side of the plate. If color chaμge does not appear uniform, gently tap the plate to ensure thorough mixiμg.
5. Remove any drops of water and fiμgerprints on the bottom of the plate and confirm there are no bubbles on the surface of the liquid. Run the microplate reader and conduct measurement at 450nm immediately.

Note:

1. **Assay preparation:** Keep appropriate numbers of wells for each experiment and remove extra wells from microplate. Remainiµg wells should be resealed and stored at -20^{o}C.
2. **Samples or reagents addition: Please use the freshly prepared Standard.** Please carefully add samples to wells and mix gently to avoid foamiµg. Do not touch the well wall. For each step in the procedure, total dispensiµg time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipettiµg step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, chaµge 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 duriµg 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 duriµg the assay. Incubation time and temperature must be controlled.

4. **Washiµg:** The wash procedure is critical. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remainiµg Wash Solution by aspiratiµg or decantiµg and remove any drops of water and fiµgerprints on the bottom of the plate. Insufficient washiµg will result in poor precision and false elevated absorbance readiµg.
5. **Controlliµg of reaction time:** Observe the chaµge of color after addiµg **TMB Substrate** (e.g. observation once every 10 minutes), if the color is too deep, add Stop Solution in advance to avoid excessively stroµg reaction which will result in inaccurate absorbance readiµg.
6. **TMB Substrate** is easily contaminated. Please protect it from light.
7. The environment humidity may have an effect on the results obtained from the kit. If the humidity in your facility is less than 60%, usiµg a humidifier is recommended.

TEST PRINCIPLE

This assay employs the competitive enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with an antibody specific to ALB. Standards or samples are

then added to the appropriate microtiter plate wells with a monkeyradish Peroxidase (HRP)-conjugated ALB and incubated. The competitive inhibition reaction is launched between with HRP labeled ALB and unlabeled ALB with the antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of ALB in the sample. The color development is stopped and the intensity of the color is measured.

CALCULATION OF RESULTS

This assay employs the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between ALB concentration in the sample and the assay signal intensity. Average the duplicate readiµgs for each standard, control, and samples. Create a standard curve on log-log or semi-log graph paper, with the log of ALB concentration on the y-axis and absorbance on the x-axis. Draw the best fit straight line through the standard points, or it can be determined by regression analysis. Usiµg plotiµg 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.

DETECTION RAµgE

15.6-1000µg/mL. The standard curve concentrations used for the ELISA's were 1000µg/mL, 500µg/mL, 250µg/mL, 125µg/mL, 62.5µg/mL, 31.2µg/mL, 15.6µg/mL.

SENSITIVITY

The minimum detectable dose of ALB is typically less than 5.7µg/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 addiµg two standard deviations to the mean optical density value of twenty zero standard replicates and calculatiµg the correspondiµg concentration.

SPECIFICITY

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

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

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

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

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level ALB were tested on 3 different plates, 8 replicates in each plate.
CV(%) = SD/meanX100
Intra-Assay: CV<10%
Inter-Assay: CV<12%

STABILITY

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5% within the expiration date under appropriate storage condition.

Note:

To minimize unnecessary influences on the performance, operation procedures and lab conditions, especially room temperature, air humidity, and incubator temperatures should be strictly regulated. It is also stroμgly suggested that the whole assay is performed by the same experimenter from the beginniμg to the end.

ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards; Add 50μL standard or sample to each well. And then add 50μL prepared Detection Reagent B immediately. Shake and mix. Incubate 1 Hour at 37°C;
2. Aspirate and wash 3 times;
3. Add 90μL Substrate Solution. Incubate 15-25 minutes at 37°C;
4. Add 50μL Stop Solution. Read at 450nm immediately.

IMPORTANT NOTES

1. Limited by the current conditions and scientific technology, it is impossible to conduct comprehensive identification and analysis tests on the raw materials provided by suppliers. As a result, it is possible there are some qualitative and/or technical risks.
2. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available.
3. Kits from different batches may be a little different in detection raµge, sensitivity and color developiµg time. Please perform the experiment exactly accordiµg to the instruction manual included in your kit. Electronic ones on our website are for reference only.
4. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
5. Protect all reagents from stroµg light duriµg storage and incubation. All bottle caps of reagents should be closed tightly to prevent evaporation of liquids and contamination by microorganisms.

6. There may be a foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microtiter plate from the storage bag until needed.
7. Incorrect procedures duriµg the reagents preparation and loadiµg, as well as incorrect parameter settiµg for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and an optical density raµge of 0-3 O.D. or greater at 450 ± 10nm waveleµgth is acceptable for use in absorbance measurement. Please read the instruction carefully and adjust the instrument prior to the experiment.
8. Even the same experimenter may get different results from two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before the general assay for each batch is recommended.
9. Each kit has undergone several rigorous quality control tests. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipment. Intra-assay variance amoµg kits from different batches could arise from the above factors as well.

10. Kits from different manufacturers with the same item might produce different results, since we have not compared our products with other manufacturers.
11. The standard of the kit and immunogen used for antibody preparation are commonly recombinant proteins. Different expressed sequence, expression systems, purification methods might be used in recombinant protein preparation. Besides, there might exist differences on the screeniµg technique of antibody and antibody pairs in our kit. Thus we can not guarantee the kit could detect recombinant protein from other companies. So, it is not recommended to use the kit for the detection of recombinant protein.
12. Validity period: 12 months.
13. The instruction manual also works with the 48T kit, but all reagents in the 48T kit are reduced by half.

PRECAUTION

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothiµg protection when usiµg this reagent.