Mouse Anti-Thyroid-Peroxidase Antibody (TPOAb) ELISA Kit

Catalog Number: EK20422

The kit is a competitive inhibition enzyme immunoassay technique for the in vitro quantitative measurement of TPOAb in mouse serum, plasma and 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

Tel: 1-301-446-2499 Fax: 1-301-446-2413

Email: tech@signalwayantibody.com Web: www.sabbiotech.com

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	6×1mL	Substrate A	1×7mL
Detection Solution A	1×6mL	Substrate B	1×7mL
Detection Solution B	1×6mL	Stop Solution	1×7mL
Wash Buffer (20 × concentrate)	1×15mL	Instruction manual	1

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader with 450 ± 10nm filter.
2. Precision 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 OF THE KITS

1. For unopened kit: All the reagents should be kept according to the labels on vials.Store at 2 - 8°C. Do not use past kit expiration date.
2. For opened kits: Once the kit is opened, the remaining reagents still need to be stored according to the above storage conditions. In addition, return the unused wells to the foil pouch containing the desiccant pack and reseal along 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 remaining reagents within 1 month of opening.

SAMPLE COLLECTION AND STORAGE

- **Serum** - Use a serum separator tube and allow samples to clot for two 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.
- **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.
- **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 performing the assay, bring samples to room temperature.

REAGENT PREPARATION

1. **Bring all kit** components and samples to room temperature (18-25^{o}C) before use.
2. **Standard** - The concentration of the standards are 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL, 0.31 ng/mL, 0ng/mL.
3. **Detection Solution A - Detection Solution B:** V Detection Solution A : V Detection Solution B = 1:10, Mix. A suggested dilution is 300 µL of Detection Solution A + 3 mL of Detection Solution B.
4. **Wash Solution** - Dilute 15mL of Wash Solution concentrate (20×) with 285mL of deionized or distilled water to prepare 300 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. If crystals have formed in the Wash Solution concentrate (20×), warm to room temperature and mix gently until the crystals are completely dissolved.
3. Any contaminated water or container used during reagent preparation will influence the detection result.

SAMPLE PREPARATION

1. SAB 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 test. 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 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 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 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. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal, store unused wells at 2 - 8°C.

3. Set a Blank well without any solution. Add 50 μL of Standard or Sample to per well. Add 50 μL of Detection Solution A to each well (Note: Do not add to Blank!). Mix well, Cover with the Plate sealer. Incubate for 1 hour at 37°C.
4. Aspirate each well and wash, repeating the process for a total of three washes. Wash by filling each well with Wash Buffer (250 μL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. 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.
5. Add 50 μL of Detection Solution B to each well (Note: Do not add to Blank!).Mix well and then incubate for 30 minutes at 37°C.
6. Aspirate each well and wash, repeating the process for a total of three washes. Wash by filling each well with Wash Buffer (250 μL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. 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 50 µL of Substrate A and 50 µL of Substrate B to each well, mix well. Incubate for 15 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark. Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. 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 630 nm. Subtract readings at 630 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.

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 4^{o}C.

2. **Samples or reagents addition: Please use the freshly prepared Standard.** Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall. 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 and 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 inaccurate absorbance reading.
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%, using a humidifier is recommended.

TEST PRINCIPLE

This assay employs the competitive inhibition enzyme immunoassay technique.The microtiter plate provided in this kit has been pre-coated with TPO antigen. Standards or samples are added to the appropriate microtiter plate wells with biotin-conjugated TPOAb. A competitive inhibition reaction is launched between TPOAb (Standards or samples) and biotin-conjugated TPOAb with the pre-coated TPO antigen. The more amount of TPOAb in samples, the

less antigen bound by biotin-conjugated TPOAb. After washing, Streptavidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Substrate solution is added to the wells and the color develops in opposite to the amount of TPOAb 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 TPOAb concentration in the sample and the assay signal intensity. Average the duplicate readings for each standard, control, and samples. Create a standard curve on log-log or semi-log graph paper, with the log of TPOAb 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. Using ploting 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 RANGE

0.31 ng/mL - 10 ng/mL. The standard curve concentrations used for the ELISA's were 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL, 0.31 ng/mL,0 ng/mL.

SENSITIVITY

The minimum detectable dose of TPOAb is typically less than 0.31 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 TPOAb.

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

Note:
Limited by current skills and knowledge, it is impossible to perform all possible cross-reactivity detection tests between TPOAb 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 TPOAb were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level TPOAb 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 strongly suggested that the whole assay is performed by the same experimenter from the beginning to the end.

ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards;
2. Set a Blank well without any solution. Add 50 μL of Standard or Sample to per well. Add 50 μL of Detection Solution A to each well (Note: Do not add to Blank!). Mix well, Cover with the Plate sealer，Incubate for 1 hour at 37°C.
3. Aspirate and wash 3 times;
4. Add 50 μL of Detection Solution B to each well (Note: Do not add to Blank!). Mix well and then incubate for 30 minutes at 37°C.
5. Aspirate and wash 5 times;. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add 50 μL of Substrate A and 50 μL of Substrate B to each well, mix well. Incubate for 15 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark. Avoid placing the plate in direct light.
7. 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 range, sensitivity and color developing time. Please perform the experiment exactly according 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 strong light during 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 during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at 450 ± 10nm wavelength 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 among 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. Validity period: 12 months.
12. 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 clothing protection when using this reagent.