Technical Manual

Human SNAPAP (SNAP Associated Protein) ELISA Kit

- Catalogue Code: AEKE01054
- Sandwich ELISA Kit
- Research Use Only

Contents

1. Key features and Sample Types

Aliases:

SNAPIN, SNAP-25-Binding Protein, Biogenesis of lysosome-related organelles complex 1 subunit 7, Synaptosomal-associated protein 25-binding protein

Standard:

10 ng/mL

Detection method:

Sandwich, Double Antibody

Sample Type:

Serum, Plasma and other biological fluids

Reactivity:

Human

Range:

0.16-10 ng/mL

Sensitivity:

0.066 ng/mL

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit at 4°C. Date of expiration will be on the ELISA Box label. Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit at 4°C. Date of expiration will be on the ELISA Box label. If the kit is opened, Store the whole kit at 4°C. If the kit is not used up in 1 week. Store the Pre-Coated Microplate, Standard, Biotinylated Antibody, and Streptavidin-HRP at -20°C, the rest reagents at 4°C, please used up within 6 months. If the kit is not opened, store the whole kit: 4°C(short-term storage, valid for 6 months); -20°C(long-term storage, valid for 1 year). Avoid repeated freeze-thaw cycles.

If the whole kit is stored at -20°C, place the kit at 4°C the day before the experiment.

3. Description and Principle

The Assay Genie Sandwich ELISA kit is a highly sensitive assay for the quantitative measurement of a specific analyte in the following samples: serum, blood, plasma, cell culture supernatant and other related supernatants and tissues.

How do our ELISA kits work?

The Assay Genie (enzyme-linked immunosorbent assays) assay kits are designed for the quantitative measurement of analytes in a wide variety of samples. As today's scientists demand premium quality, consistent data, Assay Genie have developed a range of sensitive, fast and reliable ELISA kit assays to meet and exceed those demands. Our assay kits use a quantitative sandwich ELISA technique and each kit comes with highly specific antibodies pre-coated onto a 96-well microtiter plate.

The test principle applied in this kit is Sandwich enzyme immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to Human SNAPAP. Standards or samples are added to the appropriate microtiter plate wells then with a biotin-conjugated antibody specific to Human SNAPAP. 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 Human SNAPAP, 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 450nm ± 10nm. The concentration of Human SNAPAP in the samples is then determined by comparing the OD of the samples to the standard curve.

4. Kit Contents

Each kit contains reagents for either 48 or 96 assays including:

No.	Component	48-Well Kit	96-Well Kit	Storage
1	ELISA Microplate (dismountable)	8 x 6	8 x 12	4°C/-20°C
2	Lyophilized Standard	1 vial	2 vials	4°C/-20°C
3	Sample Dilution Buffer	10 mL	20 mL	4°C/-20°C
4	Biotin-labeled Antibody (100x)	60 uL	120 uL	4°C
5	Antibody Dilution Buffer	6 mL	12 mL	4°C
6	HRP-Streptavidin Conjugate (100x)	60 uL	120 uL	4°C/-20°C
7	HRP Dilution Buffer	6 mL	12 mL	4°C/-20°C
8	TMB Substrate	6 mL	10 mL	4°C/-20°C (Avoid Direct Light)
9	Stop Solution	3 ml	6 ml	4°C/-20°C
10	Wash Buffer (25x)	10 mL	20 mL	4°C/-20°C
11	Plate Sealer	1 pieces	2 pieces	RT
12	Manual	1	1	

Additional materials required:

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

Precautions:

1. To identify the concentration of your target, a pilot experiment using standards and a small number of samples is recommended.

2. Ensure unopened and unused plate is kept dry to avoid contamination.

3. Before using the kit, centrifuge tubes to spin down standard & antibodies.

4. Avoid light for storage of TMB reagents.

5. Wash steps are critical for the success of the assay, deviations from wash steps may cause false positives and result in a high background.

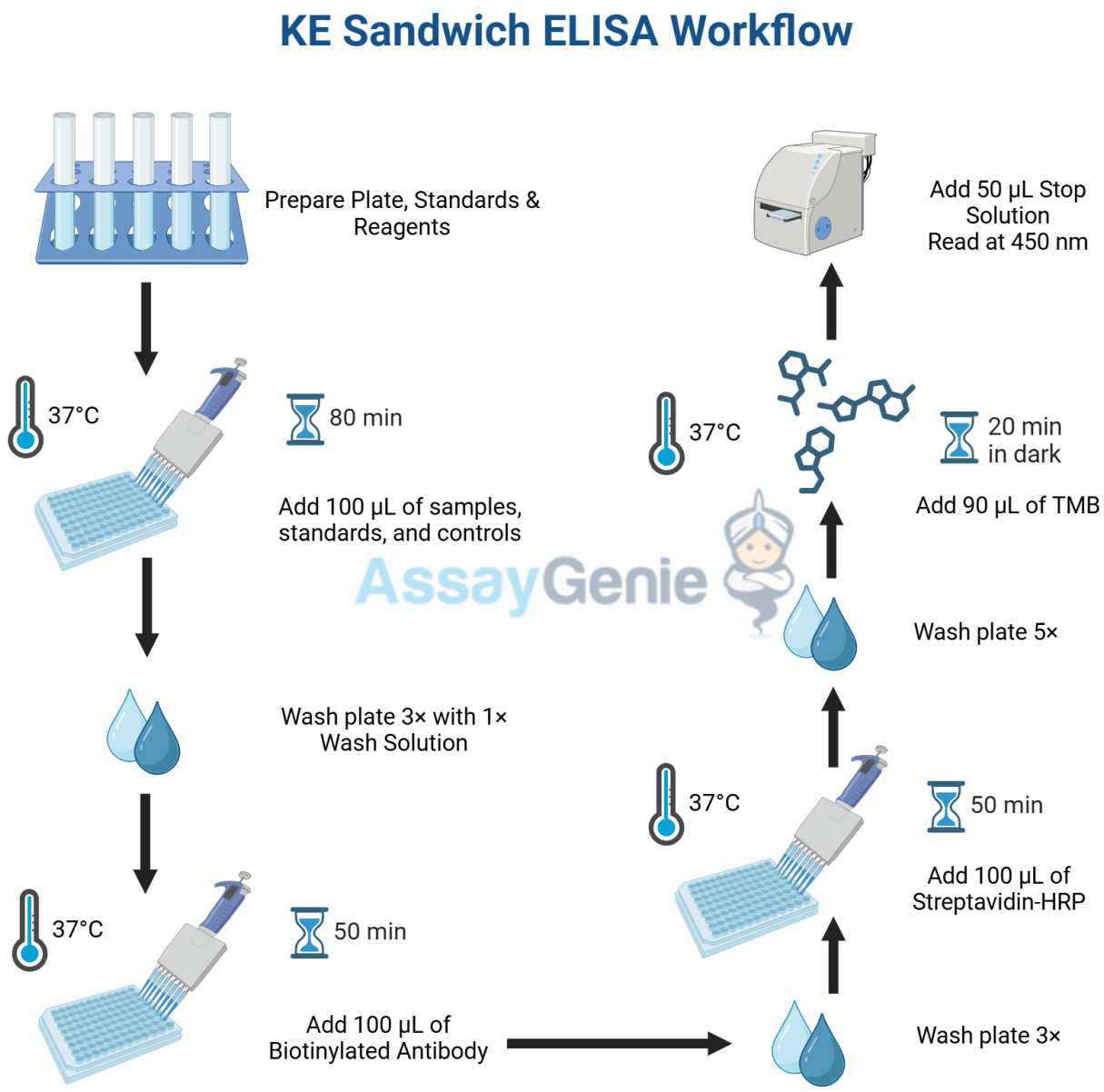
6. Duplicate wells are recommended for both standard and sample testing.

7. Do not let the microplate wells dry during assay. Dry plates will inactivate active components.

8. Do not reuse tips and tubes to avoid cross-contamination.

9. Avoid using the reagents from different batches together.

5. Workflow Overview



6. Sample Preparation

General considerations: According to best practices, extract protein & perform the experiment as soon as possible after sample collection. Alternatively, store the extracts at the designated temperature (-20°C/-80°C). For optimal results, avoid repeated freeze-thaw cycles.

Serum: Samples should be collected into a serum separator tube. After clotting for 2 hours at room temperature or overnight at 4°C, and then centrifuging at 1000 × g for 20 minutes. Assay freshly prepared serum immediately or store samples in an aliquot 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 at 1000×g and 2-8°C for 15 minutes within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

Cell culture supernatant and other biological fluids: Collect the cell culture media by pipette, followed by centrifugation at 4°C for 20 mins at 1000 x g. Collect the clear supernatant and assay immediately or store samples in an aliquot at -20°C or -80°Cfor later. Avoid repeated freeze-thaw cycles

Cell lysates: Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by pre-cooled PBS gently, and then be detached with trypsin, and collect by centrifugation at 1000 × g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells 3 times in pre-cooled PBS.
3. Then, resuspend the cells in fresh lysis buffer with a concentration of 10^7 cells/mL. If it is necessary, the cells could be subjected to ultrasonication until the solution is clear.
4. Centrifuge at 1500 × g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or store.

Tissue Homogenates: The preparation of tissue homogenates will vary depending upon tissue type.

1. Rinse the tissues in pre-cooled PBS to completely remove excess blood, and weigh them before homogenization.
2. Mince the tissues to small pieces and homogenize them in fresh lysis buffer (different lysis buffer needs to be chosen based on the subcellular location of the target protein) (PBS can be used as the lysis buffer of most tissues) (w:v = 1:9, e.g. 900 µL lysis buffer

is added in 100 mg tissue sample) with a glass homogenizer on ice (micro tissue grinders, too).

3. Ultrasound the obtained suspension with an ultrasonic cell disrupter until the solution is clear.
4. Then, centrifuge the homogenates for 5 minutes at 10000 × g and collect the supernatant and assay immediately or store it in aliquots at ≤ -20°C.

Note: Tissue homogenates are recommended to be tested for protein concentration at the same time to obtain a more accurate concentration of the test substance per mg of protein. For protein detection, you can purchase a BCA Protein concentration determination kit.

Urine: Collect the first urine of the day (mid-stream) and discharge it directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot, and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Saliva: Collect saliva using a collection device or equivalent. Centrifuge samples at 1000 × g at 2-8°Cfor15 minutes. Remove particulates and assay immediately or store samples in an aliquot at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Feces: Dry feces were collected as much as possible, weighing more than 50 mg. The feces were washed three times with PBS (w:v = 1:9, e.g. 900 µL lysis buffer is added in 100 mg feces), sonicated(or mashed), and centrifuged at 5000×g for 10 minutes, where the supernatant was collected for testing.

Cerebrospinal fluid (CSF): Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

Sample Dilution Guidelines

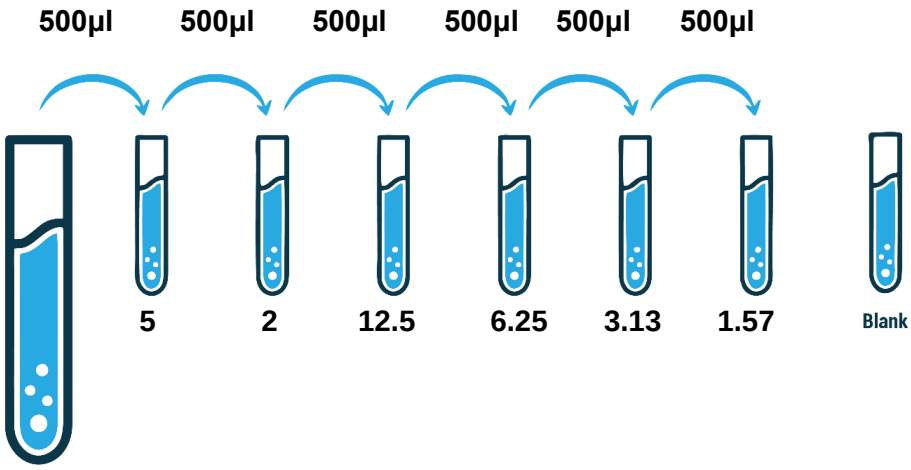
Equilibrate all materials and prepare reagents to room temperature prior to use. Before use, mix all reagents thoroughly taking care not to create any foam within the vials. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance. 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

7. Standard and Reagent Preparation

Please read the following instructions and the notes below carefully to ensure the correct preparation of the reagents:

1. **Bring all kit components and samples to room temperature (18-25°C) before use**. Make sure all components are dissolved and mixed well before using the kit. If the kit will not be used up in 1 time, please only take out strips and reagents for present experiment, and save the remaining strips and reagents as specified.
2. **Prepare Wash buffer:** Dilute the 25× Wash Buffer into 1× Wash Buffer with double-distilled Water.
3. **Standard Dilution**: Centrifuge the Standard at 1000 × g for 1 minute. ReconstitutetheStandard with 1.0 mL of Standard Diluent Buffer, keep for 10 minutes at room temperature, and shake gently (not to foam). The concentration of the Standard in the stock solution is 100 ng/mL. Please prepare 7 tubes containing 0.5 mL Standard Diluent Buffer and use the Diluted Standardtoproducea double dilution series according to the picture shown below. To mix each tube thoroughly before the next transfer, pipette the solution up and down several times. Set up 7 points of DilutedStandard such as 100 ng/mL, 50 ng/mL, 25 ng/mL, 12.5 ng/mL, 6.25 ng/mL, 3.13 ng/mL, 1.57ng/mL, and the last EP tubes with Standard Diluent is the Blank as 0 ng/mL. In order to guarantee the experimental results validity, please use the new Standard Solution for each experiment. When diluting the Standard from high concentration to low concentration, replace the pipette tip for each dilution. Note: the last tube is regarded as a Blank and do not pipette solution into it from the former tube.

DILUTION SERIES



4. **Preparation of biotin-labeled Antibody working solution Streptavidin-HRP**: Briefly spin or centrifuge the stock Biotinylated Antibody and Streptavidin-HRP before use. Dilute them to the working concentration 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively.
5. **TMB Substrate Solution:** Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Notes:

1. After receiving the kit, **please store the reagents according to the instructions**. The plates can be disassembled to single strips. Please use it in batches on demand.
2. The **test tubes, pipette tips and reagents used in the experiment are all disposable** and are strictly prohibited from being reused; otherwise, the experiment results will be affected. Kit reagents of different batches cannot be mixed (except TMB, Washing Buffer and Stop Reagent).
3. **Lyophilized Standards, Biotinylated Antibody, and Streptavidin-HRP** are small in volume and may be scattered in various parts of the tube during transportation. Please **centrifuge at 1000×g for 1 minute before use**. Then, carefully pipette 4-5 times to mix the Solution. Please **configure the Standard, Biotinylated Antibody and Streptavidin-HRP Working Solution according to the required amoun**t, and use the corresponding Dilution Solution, which cannot be mixed used.
4. **Bring all reagents to room temperature (18-25°C) before use**. If crystals form in the concentrate (25×), it is a normal phenomenon. Heat it to room temperature (the heating temperature should not exceed 40°C), gently Mix until crystals are completely dissolved.
5. Prepare to **dissolve Standard within 15 minutes before the test**. This Standard Working Solution can only be used once. If the dissolved Standard is not used up, please discard it. The sample addition needs to be rapid. Each sample addition should preferably be controlled within 10 minutes. To ensure experimental accuracy, it is recommended to test duplicate wells, and when pipetting reagents, keep a consistent order of additions from 1 well to another, this will ensure the same incubation time for all wells.
6. **During the washing process, the residual washing liquid in the reaction well should be patted dry on absorbent paper**. Do not put the paper directly into the reaction well to absorb water. Before reading, pay attention to removing the residual liquid and fingerprints at the bottom, so as not to affect the microplate reader reading.
7. **TMB Substrate Solution is light-sensitive**, avoid prolonged exposure to light. Dispense theTMBSubstrate Solution within 15 minutes following the washing of the microtiter plate. In addition, avoid contact between TMB Substrate Solution and metal

to prevent color development. Contaminated if it turns blue in color before use and should be discarded. TMB is toxic, avoid direct contact with hands.

8. **Bacterial or fungal contamination** of either samples or reagents or cross-contamination, between reagents **may cause erroneous results.**

8. Assay Procedure

1. **Determine wells for Diluted Standard, Blank and Sample.** Prepare 7 wells for Standard, 1 well for Blank. Add 100 µL each of Standard Working Solution (please refer to Reagent Preparation), or 100µL of samples into the appropriate wells. Cover with the Plate Cover. Incubate for 80 minutes at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. **Sample loading and washing:** Pour out the liquid of each well. Aspirate the solution and wash with 200 µL of 1× Wash Solution to each well and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper. Notes: (a) When adding the Washing Solution, the pipette tip should not touch the wall of the well to avoid contamination. (b) Pay attention to pouring the washing liquid directly to ensure that the washing liquid does not contaminate other wells.
3. **Add 100 µL of Biotinylated Antibody Working Solution to each well**, cover the wells with the Plate Cover, and incubate for 50 minutes at 37°C.
4. **Repeat the aspiration and wash process** for a total 3 times as conducted in step 2.
5. **Add 100 µL of Streptavidin-HRP Working Solution to each well**, cover the wells with the plate sealer and incubate for 50 minutes at 37°C.
6. **Repeat the aspiration, and wash process** for total 5 times as conducted in step 2.
7. **Add 90 µL of TMB Substrate Solution to each well.** Cover with a new Plate Cover. Incubate for 20 minutes at 37°C (Don't exceed 30 minutes) in the dark. The liquid will turn blue by the addition of TMB Substrate Solution. Preheat the Microplate Reader for about 15 minutes before OD measurement. 8. Add 50 µL of Stop Reagent to each well. The liquid will turn yellow by the addition of StopReagent.Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. The insertion order of the Stop Reagent should be the same as that of the TMB Substrate Solution. 9. Wipe off any drop of water and fingerprint on the bottom of the plate and confirm there is no bubbles on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450 nm immediately

9. Data Analysis

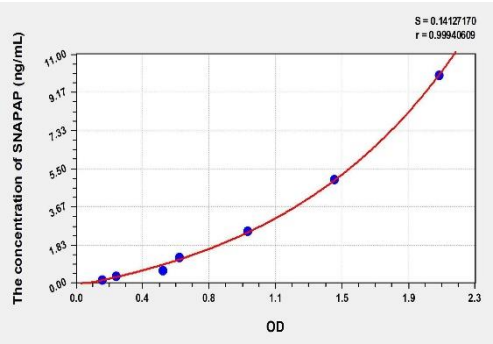
Average the duplicate readings for each Standard, Control, and Samples and subtract the average zero Standard optical density. Construct a Standard curve with the Human FN concentration on they-axis and absorbance on the x-axis, and draw a best fit curve through the points on the graph. If samples have been diluted, the concentration read from the Standard curve must be multiplied by the dilution factor. Using some plot software, for instance, curve expert.

10. Typical Data & Standard Curve

Standard Curve

Results of a typical standard run of a 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.

Concentration (ng/ml)	OD	Corrected OD
10.00	2.153	2.056
5.00	1.562	1.465
2.50	1.073	0.976
1.25	0.682	0.585
0.63	0.591	0.494
0.32	0.326	0.229
0.16	0.249	0.152
0.00	0.097	0.000



Recovery

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

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	95-107%	101%
EDTA Plasma (n=5)	80-95%	87%
Heparin Plasma (n=5)	80-93%	86%

Linearity

The linearity of the kit was assayed by testing the samples spiked with appropriate concentration of Human SNAPAP and their serial dilutions.

Sample	1:2	1:4	1:8	1:16
Serum (n=5)	93-104%	87-96%	82-95%	78-99%
EDTA Plasma (n=5)	79-92%	89-102%	87-98%	80-92%
Heparin Plasma (n=5)	95-103%	90-99%	87-95%	89-97%

Precision

- **Intra-Assay**: CV<8%
- **Inter-Assay:** CV<10%

11. Declaration

The kit may not be suitable for special experimental samples where the validity of the experiment itself is uncertain, such as gene knockout experiments. Certain natural or recombinant proteins, including prokaryotic and eukaryotic recombinant proteins, may not be detected because they do not match the detection antibody and capture antibody used in this

product. This kit is not compared with similar kits from other manufacturers or products with different methods to detect the same object, so inconsistent test results cannot be ruled out.

12. ELISA Troubleshooting

If the ELISA result is unsatisfied, please take a screenshot for the staining result and store the OD data. Keep used strips as well the rest reagents. Contact us to solve your problem with the kit's catalogue number and batch number. You can also refer to the following table to check the reason.

High Background/Non-Specific Staining

Description of results	Possible reason	Recommendations and precautions
After termination, the whole plate results show a uniform yellow or light color; or the Standard curve is linear but the background is too high	The yellowing of the whole plate may be caused by wrong addition of other reagents	Check the components and lot numbers of the reagents before the experiment, and confirm that all components belong to the corresponding kit. Reagents from different kits or different lot numbers cannot be mixed.
	ELISA plate was not washed sufficiently	Make sure that the same amount of Washing Solution is added to each microwell during the washing process. After washing, press the ELISA plate firmly on the absorbent paper to remove the residual buffer.
	Incubation time too long	Please strictly follow the steps of the manual
	Streptavidin-HRP contaminates the tip and TMB container or positive control contaminates the Pre-coated Microplate	When absorbing different reagents, the tips should be replaced. When configuring different reagent components, different storage vessels should be used. Please use a pipette during operation.
	Biotinylated Antibody or Streptavidin-HRP concentration too high	Check whether the concentration calculation is correct or use after further dilution.
	Substrate exposure or contamination prior to use	Store in the dark at all times before adding substrate.
	Color development time is too long	Please strictly follow the steps of the manual.
	The wrong filter was used when the absorbance value was read	When TMB is used as the substrate, the absorbance should be read at 450 nm.

No Color Plate

Description of results	Possible reason	Recommendations and precautions
After the color development step, all wells of the ELISA plate are colorless; the positive control is not obvious	Mixed use of component reagents	Please read labels clearly when preparing or using
	In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the color developing agent	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN3, etc.), and confirm that the container for preparing the Wash Solution has been washed.
	Missing a reagent or a step	Review the manual in detail and strictly follow the operating steps

Light Color

Description of results	Possible reason	Recommendations and precautions
The Standard is normal, the color of the sample is light	The sample uses NaN3 preservative, which inhibits the reaction of the enzyme	Samples cannot use NaN3
	The sample to be tested may not contain strong positive samples, so the result may be normal	In case of doubt, please test again.
The visual result is normal, but the reading value of the microplate reader is low	Wrong filter used for absorbance reading	When TMB is used as the substrate, theabsorbance should be read at 450 nm.

Description of results	Possible reason	Recommendations and precautions
All wells, including Standard and Samples, are lighter in color	Insufficient incubation time Timer accurate timing	Insufficient incubation time Timer accurate timing
	The number of washings increases, and the dilution ratio of the concentrated lotion does not meet the requirements Reduce the impact of washing, dilute the concentrated lotion and washing time according to the manual, and accurately record the washing times and dosage	The number of washings increases, and the dilution ratio of the concentrated lotion does not meet the requirements Reduce the impact of washing, dilute the concentrated lotion and washing time the manual, and accurately record the washing times and dosage
	Distilled water quality problem The prepared lotion must be tested to see if the pH value is neutral.	Distilled water quality problem The prepared lotion must be tested to see if the pH value is neutral.
	In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the color developing agent. Confirm that the container holding the ELISAplatedoes not contain enzyme inhibitors (such as NaN3, etc.), confirm that the container for preparing theWashing Solution has been washed, and confirm that the purified water for preparing the WashingSolution meets the requirements and is not contaminated	In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the color developing agent. Confirm that the container holding the ELISAplatedoes not contain enzyme inhibitors (such as NaN3, etc.), confirm that the container for preparing theWashing Solution has been washed, and confirm that the purified water for preparing the WashingSolution meets the requirements and is not contaminated
	The kit has expired or been improperly stored.	Please use it within the expiration and store it inaccordance with the storage conditions recommended in the manual to avoid contamination.

Description of results	Possible reason	Recommendations and precautions
	Reagents and samples are not equilibrated before use All reagents and samples should be equilibrated at room temperature for about 30 minutes.	Reagents and samples are not equilibrated before use All reagents and samples should be equilibrated at room temperature for about 30 minutes.
	Insufficient suction volume of the pipette, too fast discharge of pipetting suction, too much liquid hanging on the inner wall of the tip or the inner wall is not clean.	To calibrate the pipette, the tips should be matched, each time the tips should fit tightly, the pipetting should not be too fast, and the discharge should be complete. The inner wall of the tips should be clean, and it is best to use it once.
Poor repeatability	Incubation temperature constant temperature effect is not good.	Keep the temperature constant to avoid the local temperature being too high or too low
	When adding liquid, too much remains on the medial wall of wells.	When adding liquid, the tip should try to add liquidalong the bottom of the medial wall of wells without touching the bottom of the hole.
	Reuse of consumables	The tips should be replaced when different reagents are drawn, and different storage vessels should be used when configuring different reagent components.
	The bottom of the microwell is scratched or there is dirt	Be careful when operating, be careful not to touch the bottom and wipe the bottom of the microplate to remove dirt or fingerprints.
		Technical repetition of the same sample for 3 times, including more than 2 approximate values.
	Cross-contamination during sample addition	Try to avoid cross-contamination when adding sample

Description of results	Possible reason	Recommendations and precautions
The color of plate is chaotic and irregular	The liquid filling head of the plate washer is blocked, resulting in unsatisfactory liquid addition or large residual amount of liquid suction, resulting in the color of plate is chaotic and irregular	Unblock the liquid addition head, so that each well is filled with washing liquid when washing the plate and the residual amount should be small when aspirating liquid.
	Incomplete centrifugation of the sample, resulting in coagulation in the reaction well or interference of sediment or residual cellular components	Serum plasma should be fully centrifuged at 3000 rpm for more than 6 minutes
	The sample is stored for too long time, resulting in contamination	Samples should be kept fresh or stored at low temperature to prevent contamination
	Incorrect preparation of Washing Solution or direct misuse of concentrated Washing Solution	Please configure according to the manual

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@assaygenie.com

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