TECHNICAL MANUAL

Custom ELISA Kit (Preliminary Version)

- **SKU CODE:** SBRS6181
- **SIZE:** 96 Wells
- **DETECTION PRINCIPLE:** Sandwich ELISA
- **RUO:** Research-Use-Only

Revised: 11/26

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Please read entire manual carefully before starting experiment. DO NOT mix reagents and use reagents from different kits or batches to prevent assay failure.

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1. Key Features

Detection Method:

Sandwich

Sample Type:

Plasma, Serum

Range:

-

Sensitivity:

-

2. Storage & Expiry

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. Date of expiration is on the ELISA Box label. For prepared reagent storage, see the Materials Provided table.

3. Product Description

This assay employs a capture antibody specific for the target protein coated on a 96-well plate. Standards and samples are pipetted into the wells and the target protein present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated specific detection antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of target protein bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Important: *These kits need a 5–7 week development phase, which starts once we receive your order. Development only happens once: the finished kit must pass Assay Genie's ELISA quality control tests, and after that it becomes a stock kit with normal lead times.*

4. Materials Provided

Component	Size / Description	Storage After Preparation
Microplate*	96 wells (12 strips x 8 wells) coated with capture antibody.	1 month at 4°C*
Standard Protein	2 vials of standard protein. 1 vial is enough to run each standard in duplicate.	1 week at -80°C
Detection Antibody	2 vials of biotinylated detection antibody. Each vial is enough to assay half the microplate.	5 days at 4°C
Wash Buffer	25 mL of 20X concentrated solution.	1 month at 4°C
HRP-Streptavidin	200 µL concentrated HRP-conjugated streptavidin.	Do not store and reuse.
TMB One-Step Substrate Reagent	12 mL of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution.	N/A
Stop Solution	8 mL of 0.2 M sulfuric acid.	N/A
Assay Diluent(s)	Diluents for samples, standard and HRP-Streptavidin	1 month at 4°C

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

5. Other Supplies 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.

6. Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
3. **Sample dilution:** Samples should be diluted with provided Assay Diluent(s).

Note: *Levels of target protein may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.*

4. Preparation of standard: Appropriate standard preparation will be determined during the development process.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Zero Std
Diluent volume	Standard +							
Conc.								0

5. If the Wash Buffer (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

6. Appropriate Detection Antibody preparation will be determined during the development process.

7. Briefly spin the HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted with Assay Diluent.

7. Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Label removable 8-well strips as appropriate for your experiment.
3. Add 100 µL of each standard (see Reagent Preparation step 4) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.
4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel pipette 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 100 µL of 1X prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
6. Discard the solution. Repeat the wash as in step 4.
7. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
8. Discard the solution. Repeat the wash as in step 4.
9. Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
10. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

8. Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using SigmaPlot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

9. Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Briefly centrifuge the standard protein and dissolve the powder thoroughly by gently mixing
Low signal	Improper preparation of standard and/or biotinylated antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly.
	Too brief incubation times	Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4°C with gentle shaking (note: may increase overall signals including background).
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
	Air bubbles in wells	Remove bubbles in wells
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your standard at <-70°C after reconstitution, others at 4°C. Keep substrate solution protected from light.
	Stop solution	Add stop solution to each well before reading plate

Notes:

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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.