TECHNICAL MANUAL

Rhesus Macaque uPA ELISA Kit

- **SKU CODE:** SBRS7283
- **SIZE:** 96 Wells
- **DETECTION PRINCIPLE:** Sandwich ELISA
- **RUO:** Research-Use-Only

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Rhesus Macaque uPA ELISA Kit

Please read entire manual carefully before starting experiment.

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1. Product Description

The Assay Genie Rhesus Macaque uPA ELISA Kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of rhesus macaque uPA (urokinase-type plasminogen activator) in biological samples such as **serum, plasma and cell culture supernatants**. This assay employs a capture antibody specific for rhesus macaque uPA coated on a 96-well plate. Standards and samples are pipetted into the wells and any target protein present in the sample is bound to the wells by the immobilized antibody. The wells are washed and a biotinylated antibody specific for rhesus macaque uPA is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted into 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.

2. Precision

- **Intra-Assay CV%:** <10%
- **Inter-Assay CV%:** <12%

3. Storage

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. For the storage of prepared reagents, see the table in Section 4.

4. Materials Provided

Component	Size / Description	Storage / Stability After Preparation
Rhesus Macaque uPA Microplate	96 wells (12 strips x 8 wells) coated with anti-rhesus macaque uPA.	1 month at 4°C*
Rhesus Macaque uPA Standard Protein	2 vials of rhesus macaque uPA standard protein. 1 vial is enough to run each standard in duplicate.	1 week at -80°C
Rhesus Macaque uPA Detection Antibody	2 vials of biotinylated anti-rhesus macaque uPA. 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 of 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 the desiccant pack and reseal along the 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 the 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:** Prepare the standard dilution series as described in the dilution instructions supplied with the kit. The standard is prepared as a serial dilution, as illustrated below; the final tube serves as the zero standard and contains diluent only.
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. Prepare the Detection Antibody as described in the instructions supplied with the kit.
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.

	Std 1	**Std 2**	**Std 3**	**Std 4**	**Std 5**	**Std 6**	**Std 7**	**Blank**
Diluent volume	Standard + µL	µL	µL	µL	µL	µL	µL	µL
Conc.								

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 (see 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 suitable curve-fitting 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 · Improper standard dilution	Check pipettes. Briefly centrifuge the standard protein and dissolve the powder thoroughly by gently mixing.
Low signal	Improper preparation of standard and/or biotinylated antibody · Too brief incubation times · Inadequate reagent volumes or improper dilution	Briefly spin down vials before opening. Dissolve the powder thoroughly. 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). Check pipettes and ensure correct preparation.
Large CV	Inaccurate pipetting · Air bubbles in wells	Check pipettes. Remove bubbles in wells.
High background	Plate is insufficiently washed · Contaminated wash buffer	Review the manual for proper washing. If using a plate washer, ensure that all ports are unobstructed. Make fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit · Stop solution	Store your standard at <-70°C after reconstitution, others at 4°C. Keep substrate solution protected from light. Add stop solution to each well before reading the plate.

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.