Dilution Buffer (Strengthen Blocking)

Cat. No: DB-02
Pack Size :50mL/500mL

For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedures

Applications

Serum and some samples often cause nonspecific binding, especially after blocking, which lead to high background and low sensitivity. The buffer can reduce non-specific adsorption in experiments, avoid background signals which is caused by serum especially, achieve high signal-to-noise ratio, and achieve ideal detection sensitivity.

It is for research use only.

Shipping and Storage

Upon receipt, please store at 2-8°C, Once opened, store at 2-8°C.

Example ELISA Procedure

1. Materials and Reagents Preparation

Before starting the ELISA Assay, we should prepare the all reagents and materials required in the experiment. You can prepare these regents by following operations, **we provide the Dilution Buffer (Strengthen Blocking) (Cat. No. DB-02).**

Wash Buffer: PBS or TBS with 0.05% (v/v) Tween-20 (usually at pH7.4), 500 mL is sufficient for 96 tests. The pH of Buffer system can be adjust according to your experiment.

Substrate Dilution Buffer: 50 mM disodium hydrogen phosphate (Na_2HPO_4) and 25 mM citric acid, adjust pH to 5.5 with 1 M Sodium hydroxide (NaOH), 25 mL is sufficient for 96 tests.

Substrate Stock Solution: 20 mg/mL TMB (Sigma-Aldrich, Catalog # 860336) in Dimethyl sulfoxide (Sigma-Aldrich, Catalog # D8418), 1 mL is sufficient for 96 tests. Protect from light TMB.

Substrate Working Solution

For each plate dilute 125 μL substrate stock solution in 25 mL substrate dilution buffer and add 20 μL 5% H_2O_2 (pipette 10 μL 30% H_2O_2 into 50 μL distilled water), mix well.

Notes:

1) The TMB Substrate Working Solution should be freshly prepared and used within 15 minutes.

2) If you choose to use other commercially available ready-to-use TMB substrate solutions, you should follow the manufacturer's instruction.

Stop Solution: 1 M sulfuric acid (aqueous), 6 mL is sufficient for 96 tests.

Coated Plates (ACROBiosystems, Catalog # SP-11)

Microplate sealing film (Sigma-Aldrich, Catalog # Z724742)

Pipettes and pipette tips

UV/Vis microplate spectrophotometer (absorbance 450 nm, correction wavelength set to 630 nm)

2. Recommended Protocol

2.1 Preparation

Reconstitute and store all reagents as recommended.

2.2 Washing

Add 300 μL of Wash buffer to each well, gently tap the plate for 1 minute, remove any remaining Wash Buffer by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

2.3 Add biotinylated protein or antibodies

1) Dilute Biotinylated protein or antibodies to a concentration you want (usually 1~10 μg/mL) with Dilution Buffer to make Biotinylated molecule working solution.

2) Add 100 μL Biotinylated molecule to each well and incubate at 37°C or RT for 1 hour.

3) For Non - specific of the sample wells, please add 100 μL Dilution Buffer.

2.4 Washing

Remove the remaining solution by aspiration, add 300 μL of Wash buffer to each well, gently tap the plate for 1 minute, remove any remaining Wash Buffer by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

2.5 Add Samples

1) Make series dilution of the samples as appropriate with Dilution Buffer.

2) Add 100 μL of the serial dilution of sample to each well, incubate at 37°C or RT for 1 hour.

2.6 Washing

Repeat step 4.

2.7 Add primary antibody

1) Dilute primary antibody to an appropriate concentration with Dilution Buffer.

2) For all wells, add 100 µL of diluted primary antibody, and incubate at 37°C or RT for 1 hour.

2.8 Washing

Repeat step 4.

2.9 Add enzyme-labeled secondary antibody

1) Dilute secondary antibody to an appropriate concentration with Dilution Buffer.

2) For all wells, add 100 µL of diluted secondary antibody, and incubate at 37°C or RT for 1 hour, avoid light.

2.10 Washing

Repeat step 4.

2.11 TMB Substrate Reaction

Add 200 µL TMB Substrate Working Solution to each well. Seal the plate with microplate sealing film and incubate at 37°C or RT for 20 minutes, avoid light.

2.12 Termination

Add 50 µL Stop Solution to each well, and tap the plate gently for 3 minutes to allow thorough mixing.

Note: *the color in the wells should change from blue to yellow.*

2.13 Data Recording

Read the absorbance at 450 nm using UV/Vis microplate spectrophotometer.

Note: *the plate may be read at 600 nm without adding 1 M sulfuric acid, but the Signal-to-Background ratio.*

3. Example Data

Binding Assay between S protein RBD and Anti-SARS-CoV on SA Plate

Immobilized Biotinylated SARS-CoV-2 S protein RBD, His, Avi tag at 1000ng/mL (100 μL/well) on Streptavidin Coated Plates, Clear, 96-Well (Cat. No. SP-11), can bind Anti-SARS-CoV antibody with a linear range of 0.1-3 ng/mL.