Streptavidin Coated Plates, Clear, 96-Well, Clear Frame

(For Serological Testing)

Cat. No: SP-15
Pack Size :1 Plate
For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedures.
IMPORTANT: Please carefully read this manual before performing your experiment.

Specifications

Table1. plate details

Items	Specifications
Material	**Polystyrene**
Color	Clear
Plate Blocking	2% BSA Blocking Buffer
Formulations	Clear, 96-Well, Clear Frame, coated with 100 µL of streptavidin tetramerand blocked with 200 µL of 2% BSA Blocking Buffer，Dilution Buffer 50 mL
Detection Method	Colorimetric
Capacity	~5 pmol biotin/well
CV% of plates/wells	< 10%
Type	Detection Plate, Immunoassay, ELISA

Shipping and Storage

Upon receipt, please store all items at 2-8°C. This plate is supplied and shipped with blue ice.

Product description

Streptavidin Coated Plates, Clear, 96-Well, Clear Frame (For Serological Testing) with Streptavidin tetramer Protein and blocked with BSA, it is a ready-to-use polystyrene plate as well as a dilution buffer containing serum samples, which can be used for binding biotinylated proteins and antibodies, or probes for ELISA and other target specific assays. The recombinant Streptavidin is tetramer protein expressed in E. coli designed for immobilization applications.

Applications

The Streptavidin Coated Plate is developed for immunoassay and ELISA of serum samples.

It is for research use only.

Assay Principles

Streptavidin (SA) has an extraordinarily high affinity for biotin with a dissociation constant (Kd) on the order of 10^{-14} mol/L, the Biotinylated molecules can bind to the SA irreversibly. Streptavidin has an isoelectric point of 5

to 6, resulting in low nonspecific interactions. The Streptavidin Coated Plates we provide are easy to use and widely available for application.

Example ELISA Procedure

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 also provide the matching reagent kit (Cat. No. SP-15).

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.

Dilution Buffer: The kit (Cat. No. SP-15) comes with diluent Buffer， 50 mL is sufficient for 96 tests.

Substrate Dilution Buffer: 50 mM disodium hydrogen phosphate (Na_2HPO4) 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.

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

Pipettes and pipette tips

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

Recommended Protocol

1. **Preparation**

Reconstitute and store all reagents as recommended.

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.

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℃ or RT for 1 hour.
3) For Non - specific of the sample wells, please add 100 μL Dilution Buffer.

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.

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℃ or RT for 1 hour.

6. Washing

Repeat step 4.

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℃ or RT for 1 hour.

8. Washing

Repeat step 4.

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℃ or RT for 1 hour, avoid light.

10. Washing

Repeat step 4.

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℃ or RT for 20 minutes, avoid light.

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.*

13. Data Recording

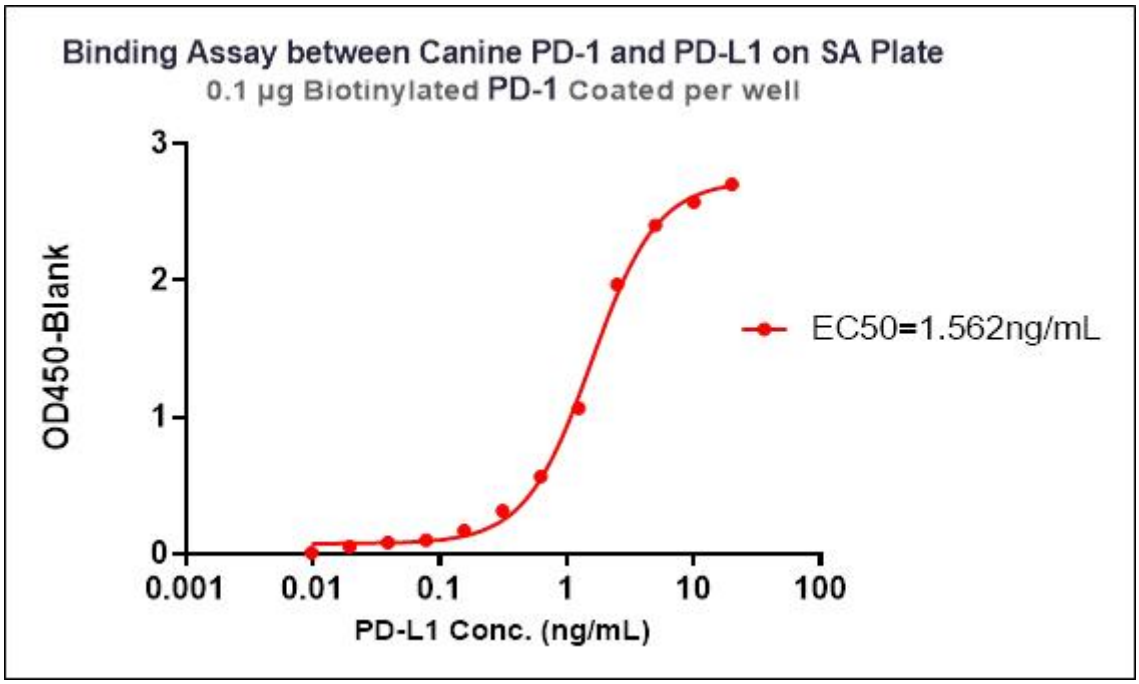
Read the absorbance at 450 nm using UV/Vis microplate spectrophotometer.

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

Example Data

Binding Assay between Biotinylated Canine PD-1 and PD-L1 on SA Plate

I Immobilized Biotinylated Canine PD-1, His&Avi tag at 1000 ng/mL (100 µL/well) on Streptavidin Coated Plates, Clear, 96-Well, Clear Frame (For Serological Testing) (Cat. No. SP-15), can bind PD-L1 with a linear range of



0.01-20ng/mL (QC Report)

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solutions
Signal of positive control is weak or abnormal	**Incorrect storage of plate**	✧ **The plate should be store plates at 4°C, once you open the package, get the amount you need and keep the rest airtight.**
	Detection Antibody is outdated or no prepared the working solution immediately before use	✧ **The working solution should be prepared immediately before use and should not be stored.**
	Errors in instrument settings	✧ Please check instrument setting.
	Substrate Stock Solution is outdated; Incubation temperature is incorrect; Incubation time is not sufficient; Repeated freeze-thaw cycles;	✧ Make sure the **Substrate Stock Solution** is working. ✧ Use proper incubation time and temperature.
	Pipetting errors	✧ Make sure that the pipette is calibrated and working properly.
High background	Serum samples	✧ Serum dilution of 50 times to 2% is recommended
	Sample solvent contains inhibiting factors	✧ Run a negative control assay with the solvent alone. ✧ Maintain DMSO level at <1 . Increase protein incubation time.
	Contamination	Make sure buffers and samples are prepared, used and stored correctly.
	The **TMB Substrate Working Solution** is not fresh	✧ **TMB Substrate Working Solution** must be used within 15 minutes after preparation.
Colorimetric signal is erratic	nconsistent pipetting or dilution methods	✧ Make sure pipettors are functioning properly and use a multichannel pipettor if possible. ✧ Use master mixes to minimize errors. ✧ Run duplicates for all tests.
	TMB Substrate Working Solution is not completely mixed with the reaction solution	✧ Make sure that **TMB Substrate Working Solution** is adequately mixed with the reaction solution.
	Bubbles in the wells	✧ Tap plate gently to disperse bubbles.
	Signal is too high	✧ The concentration of the samples should be adjusted to achieve optimal reading. ✧ Decrease colorimetric HRP substrate incubation time.
Inadequate color development	Incomplete removal during previous steps of residual buffers	✧ Wells should appear dry after aspiration.
	Problems with conjugate or color reagents	✧ Color should appear immediately after the reagent is added. Make sure no contamination or residual buffers in the wells before you start the color development process.