Nickel Coated Plates, Clear, 12×8-Well Strips, White Frame

Cat. No: SP-19 **Pack Size :1 Plate**

Important: Please carefully read this manual before performing your experiment.

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

SPECIFICATIONS

Table1. plate details

Items	Specifications
Material	Polystyrene
Color	Clear
Formulations	Clear, 96-well plates, coated with Ni2+
Detection Method	Colorimetric
Capacity	~4.5 pmol His-Tag protein(26.6kDa)/well(100μL)
CV% of plates/wells	＜10%
Type	Detection Plate, Immunoassay, ELISA

STORAGE

The unopened plate should be stored at 2°C to 8°C, The expiry date of the plate is 12 months. Once opened, place unused plates in a resealable bag with desiccant and store at 2°C to 8°C, The shelf life is 1 month from the date of opening.

PRODUCT DESCRIPTION

Porath first discovered in 1975 that transition state metal ions immobilized on a substrate can achieve both specific binding effects through coordination with residues such as histidine, cysteine, tryptophan on the protein surface. The metal chelate affinity technique has become a powerful tool for the research and development of biopharmaceuticals because of its high selectivity, high adsorption capacity, mild conditions, high selectivity, excellent separation efficiency, reproducibility and versatility. The Nickel coated plate, a solid-state support for the purification / enrichment / capture of histidine (His) - tagged proteins through the chelation immobilization of Ni^{2+} combined with triacetic acid nitrate (NTA), are one of the most commonly used metal chelate affinity techniques.

APPLICATIONS

The plate is developed for specifically bind His-tagged proteins and can be used for applications such as concentration quantification of protein samples, affinity validation of proteins to their receptors / antibodies based on ELISA and CLIA assay.

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-19).

1. **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.
2. **Dilution Buffer1:** 2%BSA in the wash Buffer
3. **Dilution Buffer2:** 0.5% BSA in the wash Buffer
4. **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.
5. **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.
6. **TMB Substrate Working Solution**

For each plate dilute 125 µL substrate stock solution in 25 mL substrate dilution buffer and add 15 µL 3% H_2O_2, 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.

7. **Stop Solution:** 1 M sulfuric acid (aqueous), 6 mL is sufficient for 96 tests.
8. **Microplate sealing film** (Sigma-Aldrich, Catalog#Z724742)
9. **Pipettes and pipette tips**
10. **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, 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 His-Tag Sample

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

2) Add 100 μL working solution to each well and incubate at RT for 1.5 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, 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.

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 enzyme-labeled secondary antibody

1) Dilute secondary antibody to an appropriate concentration with Dilution Buffer 2.

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

8. Washing

Repeat step 4.

9. 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.

10. 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.*

11. Data Recording

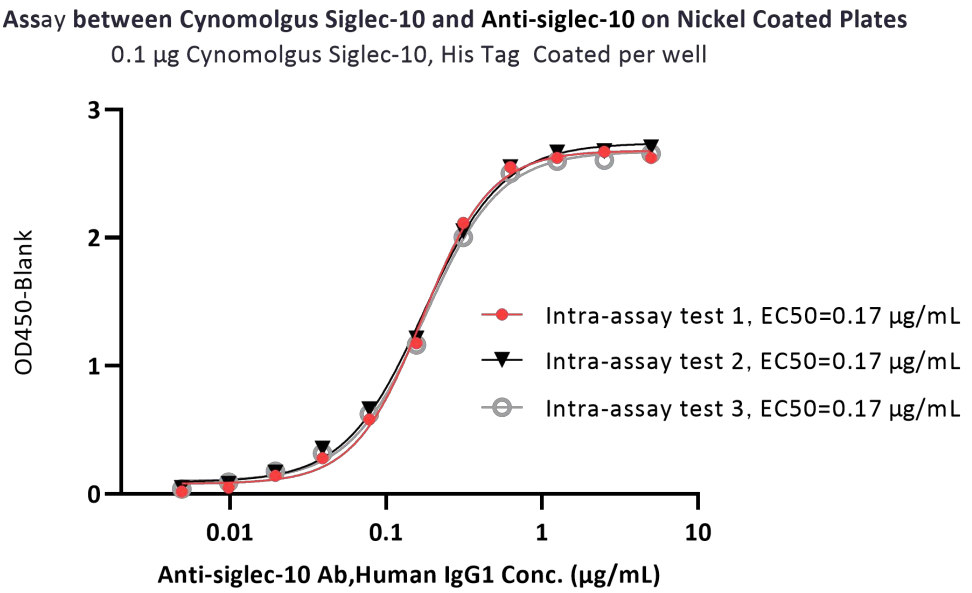
Read the absorbance at 450 nm using UV/Vis microplate spectrophotometer.

***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 Cynomolgus Siglec-10, His Tag and Anti-siglec-10 on Nickel Coated Plates



Immobilized Cynomolgus Siglec-10, His Tag at 1 μg/mL (100 μL/well) on Nickel Coated plates, Clear, 12×8-Well Strips(Cat.No.SP-19), can bind Anti-Cynomolgus Siglec-10 Antibody, Human IgG1 with a linear range of 0.005-0.313 μg/mL, CV% of Intra-assay tests < 10% (QC tested).

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solutions
Signal of positive control is weak or abnormal	**Incorrect storage of plate**	✧ **Once opened, place unused plates in a resealable bag with desiccant and store at 2°C to 8°C, The shelf life is 1 month from the date of opening.**
	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 incubationtime.
	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 15minutes 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.[i] ✧ 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.