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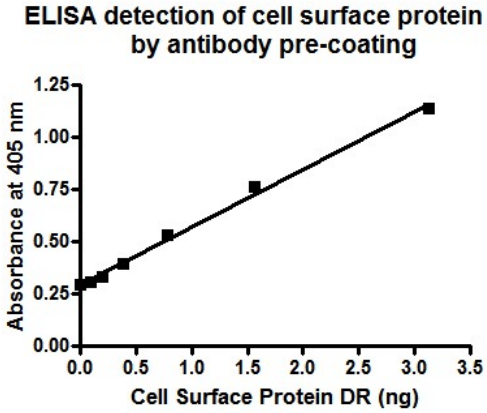
INSTRUCTIONS

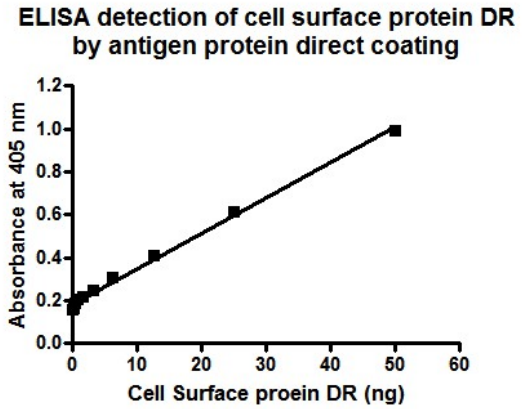
ProFoldin
96-well Super High Binding ELISA Plates

96-well Super High Binding ELISA Plates - Transparent **Catalog No. ELISA96T**
96-well Super High Binding ELISA Plates - Black **Catalog No. ELISA96B**

Introduction

The 96-well Super High Binding ELISA Plates are designed for strong signals of ELISA. The ELISA sensitivity is dramatically enhanced by strong protein binding on the plates. In a direct binding mode, the antigen is coated onto the plate first. After blocking, the antibody against the antigen is bound to the antigen and the bound antibody is detected by an enzyme-linked second antibody. Alternatively, if two antibodies (A and B) against the antigen are available, antibody A is coated onto the plate first. After blocking, the antigen is bound to the coated antibody A. Then antibody B is bound to the antigen. Finally the bound antibody B is detected by the enzyme-linked antibody.





The 96-well Super High Binding ELISA Plates – Transparent (Catalog No. ELISA96T) are for absorbance detection of the reaction catalyzed by the enzyme-linked antibody. The 96-well Super High Binding ELISA Plates – Black (Catalog No. ELISA96B) are for fluorescence detection. Each plate set includes 4 plates.

Protocol 1 – Antigen direct binding

1. **Antigen binding:** Coat the plate with 100 µl of antigen solutions with 2 fold dilution in PBS supplemented with 0.02 % sodium azide. The assay sensitivity depends on the nature of the

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antigen protein, a typical maximum antigen concentration is 1 µg /ml. Rotate the plate at 4°C overnight.

2. **Wash:** Wash the plate 4 times with 360 µl of Washing Buffer composed of PBS supplemented with 0.01 % Tween-20.
3. **Block:** Block the plate with 360 µl of Blocking Solution composed of PBS supplemented with 0.02 % sodium azide and 3% fetal bovine serum at room temperature for 2 hr.
4. **Wash:** repeat Step 2
5. **Primary antibody binding**: Add 100 µl of the antibody solution diluted in Dilution Solution composed of PBS supplemented with 0.02 % sodium azide and 0.1% fetal bovine serum and incubate the plate with constant rotating at room temperature for 1 hr.
6. **Wash:** repeat Step 2
7. **Secondary antibody binding**: Add 100 µl of the second antibody diluted in Dilution Solution. Incubate the plate with constant rotating at room temperature for 1 hr.
8. **Wash:** repeat Step 2
9. **Detection:** Add 200 µl of detection solution and read the signals the assay wells. The signal can be absorbance or fluorescence depending on the property of the secondary antibody.

Protocol 2 – with a primary antibody pre-coating

Pre-coating with a primary antibody (A) may enhance the ELISA sensitivity if a second antibody (B) against the antigen ids available.

1. **Antibody coating:** Coat the plate with 100 µl of 1 ug/ml the primary antibody (A) in PBS supplemented with 0.02 % sodium azide. Rotate the plate at 4°C overnight or 37°C for 2 hr.
2. **Wash:** Wash the plate 4 times with 360 µl of Washing Buffer composed of PBS supplemented with 0.01 % Tween-20.
3. **Block:** Block the plate with 360 µl of Blocking Solution composed of PBS supplemented with 0.02 % sodium azide and 3% fetal bovine serum at room temperature for 2 hr.
4. **Wash:** repeat Step 2
5. Follow Protocol 1 from Step 1 to Step 10 except omitting the Block step (Step 3).

Related products

APP96 T	96-well Anionic Protein-binding Plates (transparent)
NBP96B	96-well DNA-binding Plate (black)

For more information of molecular binding, separation and analysis, please visit www.profoldin.com.