INSTRUCTIONS

Pierce® Glutathione Coated 96-Well Plates

15140 15240 15340

0685.2

Number	Description
15140	**Pierce Glutathione Coated Plates (8-well strips),** 5 plates
15240	**Pierce Glutathione Coated Plates (white, 96-well),** 5 plates
15340	**Pierce Glutathione Coated Plates (black, 96-well),** 5 plates
	Capacity: ~10ng purified GST per well
	Blocking Buffer: Plates are supplied blocked with SuperBlock® Blocking Buffer
	Coating Volume: 100µL
	Blocking Volume: 200µL

Storage: Upon receipt store plates at 4°C in unopened pouches. Once opened, place unused plates in a resealable bag with desiccant and store at 4°C. Plates are shipped at ambient temperature.

Introduction

Before purification of GST-fusion proteins using glutathione-agarose beads, it is often useful to screen for the presence of GST proteins in cell lysates. The Thermo Scientific Pierce Glutathione Coated Plates have glutathione immobilized on plates through its central sulfhydryl. These plates are useful for analyzing cell lysates to determine the presence and concentration of GST-fusion protein and for screening sera for antibodies to the GST-fusion protein. These plates are pre-blocked, making initial purification of the cell lysates unnecessary.

General Procedure for GST-Fusion Protein Detection

A. Materials Required

- Cell lysate containing GST-tagged protein
- Dilution buffer: Phosphate-buffered saline (PBS; Product No. 28372) or Tris-buffered saline (TBS; Product No. 28376)
- Wash Buffer: PBS or TBS with 0.05% Tween®-20 Detergent (Product No. 28320)
- Anti-GST antibody, 1mg/mL (Product No. 30001) or antibody against the protein of interest
- Enzyme- or fluorophore-conjugated secondary antibody to the species in which the anti-GST was created
- Appropriate enzyme substrate: example substrates are the Thermo Scientific TMB Substrate Kit (Product No. 34021) for horseradish peroxidase and the Thermo Scientific Phosphatase Substrate Kit (Product No. 37620) for alkaline phosphatase

B. Method

1. Rinse each well three times with 200µL of wash buffer.
2. Prepare serial dilutions of the cell lysate in wash buffer.

   Note: To determine the approximate concentration of GST-tagged protein, generate a standard curve using purified GST.
3. Apply 100µL of each lysate serial dilution to duplicate wells. Apply 100µL/well of wash buffer to separate duplicate wells to measure background. Cover the plate and incubate for 1 hour at room temperature.
4. Rinse the plate three times with 200µL/well of the wash buffer.

5. Prepare an appropriate dilution of the anti-GST antibody in dilution buffer and apply 100µL to each well. Cover the plate and incubate for 1 hour at room temperature.
6. Wash the plate three times with 200µL/well of wash buffer.
7. Prepare an appropriate dilution of the labeled secondary antibody and add 100µL to each well. Cover the plate and incubate for 1 hour at room temperature.
8. Wash the plate three times with 200µL/well of wash buffer.
9. Follow manufacturers' instructions for the specific detection system being used.

Related Thermo Scientific Products

28372	**BupH™ Phosphate Buffered Saline Packs,** 40 packs
28376	**BupH Tris Buffered Saline Packs,** 40 packs
37070	**SuperSignal® ELISA Pico Chemiluminescent Substrate,** 100mL
15169	**QuantaBlu™ Fluorogenic Peroxidase Substrate Kit**
34028	**1-Step Ultra TMB-ELISA,** 250mL
37621	**1-Step PNPP,** 100mL
15075	**Reagent Reservoirs,** 200/pkg
15082	**Microtube Racked System,** 960 tubes
15036	**Sealing Tape for 96-Well Plates,** 100/pkg

General References

Smith, D.B. and Johnson, K.S. (1988). Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with gluathione S-transferase. *Gene* **7**:31-40.

Frangioni, J.V. and Neel, B.G. (1993). Solubilization and purification of enzymatically active glutathione S-transferase (pGEX) fusion proteins. *Anal Biochem* **210:**179-87.

Simons, P.C. and VanderJagt, D.L. (1977). Purification of gluathione S-transferases for human liver by glutathione-affinity chromatography. *Anal Biochem* **82:**334-41.