

Pierce<sup>®</sup> Anti-GST Coated Plates

15144

2102.2

| Number | Description                                                                                                                                                                           |
|--------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 15144  | <b>Pierce Anti-GST Coated Plates</b> , 5 plates<br>Capacity: ~10ng purified GST per well<br><b>Note:</b> Plates are pre-blocked with SuperBlock <sup>®</sup> Blocking Buffer (200µL). |

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

### Introduction

The Thermo Scientific Pierce Anti-GST Coated Plates are ideal for determining the presence and concentration of GST or GST-fusion proteins from cell lysates. Because the plates are pre-blocked, initial purification of the cell lysates is not necessary. Also, using these plates to immobilize GST fusion proteins is useful for screening sera for antibodies to the fusion protein.

### General ELISA Procedure using the Anti-GST Coated Plates

#### A. Materials Required

- Cell lysate containing GST-tagged protein
- Dilution buffer such as phosphate-buffered saline (PBS) or Tris-buffered saline (TBS)
- Wash buffer: Dilution buffer containing 0.05% Tween<sup>®</sup>-20 Detergent (Product No. 28320)
- GST Polyclonal Antibodies (e.g., Product No. CAB4169) or an antibody against the protein of interest. Please see [www.thermoscientific.com/pierce-abs](http://www.thermoscientific.com/pierce-abs) for a complete listing.
- Enzyme-conjugated secondary antibody to the species in which the anti-GST was created
- Appropriate enzyme substrate stop solution, if required

### Assay of GST cell lysate containing the GST fusion Protein

**Note:** To determine the approximate concentration of the GST-tagged protein, generate a standard curve using purified GST.

1. Rinse each well three times with 200µL of wash buffer.
2. Prepare a dilution of the cell lysate in the wash buffer (suggested starting dilution is 1:100-1:1000).
3. Apply 100µL of the lysate to duplicate wells in row 1 of the microplate. Make a serial dilution of the lysate and apply 100µL of each to duplicate wells in rows 2-7. Apply 100µL/well of the wash buffer to duplicate wells in row 8 to measure background.
4. Cover the plate and incubate for 1 hour at room temperature.
5. Rinse the plate three times with 200µL/well of wash buffer.
6. Prepare an appropriate dilution of the anti-GST antibody or appropriate primary antibody in the dilution buffer (See manufacturer's instructions for proper dilution).
7. Apply 100µL/well of the primary antibody (anti-GST or other) to all wells.
8. Cover the plate and incubate for 1 hour at room temperature.

9. Wash the plate three times with 200µL/well of wash buffer.
10. Prepare an appropriate dilution of the labeled secondary antibody and add 100µL to each well.
11. Cover the plate and incubate for 1 hour at room temperature.
12. Wash the plate three times with 200µL/well of wash buffer.
13. Follow manufacturer's instructions for the particular colorimetric detection system being used.

## General References

- Janknecht, R., *et al.* (1991). Rapid and efficient purification of native histidine-tagged protein expressed by recombinant vaccinia virus. *Proc Natl Acad Sci USA* **88**: 8972-6.
- Riggs, P., In Ausubel, F.M., *et al.* Ed., (1990). *Current Protocols in Molecular Biology* 16.4.1-16.6.14.
- Smith, D.B. and Johnson, K.S. (1988). Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. *Gene* **7**: 31-40.

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|              |                                                                             |
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| <b>78248</b> | <b>B-PER Bacterial Protein Extraction Reagent, 500mL</b>                    |
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