

# Reacti-Bind™ Glutathione Coated Plates

15140 15240 15340

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## Product Description

Number	Description
15140	<b>Reacti-Bind™ Glutathione Coated Strip-Well Plates, 5 plates/package</b>
15240	<b>Reacti-Bind™ White Opaque Glutathione Coated 96-Well Plates, 5 plates/package</b> <i>Recommended for chemiluminescent detections</i>
15340	<b>Reacti-Bind™ Black Opaque Glutathione Coated 96-Well Plates, (5 plates/package)</b> <i>Recommended for fluorescent detections</i>
	<b>Detection Levels:</b> > 1 ng of purified GST per well may be detected using these plates.
	<b>Capacity:</b> ~10 ng purified GST per well.

Store plates refrigerated in unopened pouches. Place opened, unused plates in a resealable bag with desiccant and store refrigerated. These ready-to-use plates are pre-blocked to help prevent non-specific interactions.

## Introduction

The discovery that bacterial plasmid vectors may be used for the synthesis of proteins which are “fused” together has resulted in the creation of a new class of proteins known as “fusion” proteins. The fused proteins, one of which has a strong affinity for a known ligand, may be easily purified using affinity chromatography. Examples of these fusion proteins are the Glutathione-S-Transferase (GST) fusion proteins<sup>1</sup>, Maltose Binding Protein (MBP) fusion proteins<sup>2</sup> and poly-histidine tagged fusion proteins.<sup>3</sup>

Purification of GST fusion proteins using glutathione-agarose beads is well documented.<sup>4,5</sup> Prior to purification, it may be useful to screen for the presence of GST proteins in cell lysates. We have immobilized glutathione through its central sulfhydryl to the wells of these plates. These glutathione coated plates can be used in the analysis of cell lysates to determine the presence and concentration of the GST-fusion protein. Because the plates are pre-blocked, initial purification of the cell lysates is not necessary. Specific binding of the protein is achieved through the interaction of GST with glutathione.

Immobilization of GST fusion proteins using the glutathione coated microwell plates may also prove useful in the screening of sera for antibodies to the GST-fused protein. A generalized protocol for the use of the Reacti-Bind™ Glutathione Coated Plates is given below.

## Example Protocol for the Use of Glutathione Coated Plates

### Materials Required

- A. Reacti-Bind™ Glutathione Coated Plates.
- B. Cell lysate containing GST-tagged protein.
- C. Dilution buffer: e.g., Phosphate Buffered Saline (PBS) or Tris Buffered Saline (TBS).

**Note:** Use TBS if Alkaline Phosphatase labeled secondary antibody will be used for detection.

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- D. Wash Buffer: Dilution buffer containing 0.05% Tween<sup>®</sup> 20 (Pierce Prod. No. 28320).
- E. Anti-GST Antibody, 1mg/ml (Available from Pierce as Prod. No. 30001) or antibody against the protein of interest.
- F. Enzyme or fluorescent conjugated secondary antibody to the species in which the anti-GST was created
- G. Appropriate enzyme substrate, if required. A wide range of chemiluminescent and colorimetric substrates are available from Pierce.
- H. Appropriate stop solution for enzyme substrate, if required.

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

1. Rinse each well with 3 x 200  $\mu$ l 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 100  $\mu$ l of the lysate to duplicate wells in row 1 of the microwell plate. Make a serial dilution of the lysate and apply 100  $\mu$ l of each to duplicate wells in rows 2-7. Apply 100  $\mu$ l/well of the wash buffer to duplicate wells in row 8 to measure background. Cover the plate.
4. Incubate the plate for 1 hour at room temperature.
5. Rinse the plate three times with 200  $\mu$ l/well of the wash buffer.
6. Prepare an appropriate dilution of the Anti-GST antibody or appropriate primary antibody in the dilution buffer (see manufacturers instructions for proper dilution).
7. Apply 100  $\mu$ l/well of the primary antibody (Anti-GST or other) to all the wells. Cover the plate.
8. Incubate the plate for 1 hour at room temperature.
9. Wash the plate three times with 200  $\mu$ l/well of the wash buffer.
10. Prepare an appropriate dilution of the labeled secondary antibody.
11. Apply 100  $\mu$ l/well of the labeled secondary antibody to all the wells. Cover the plate.
12. Incubate the plate for 1 hour at room temperature.
13. Wash the plate three times with 200  $\mu$ l/well of the wash buffer.
14. Follow manufacturers instructions for the particular colorimetric, chemiluminescent or fluorescent detection system being used.

**Note 1:** To determine the approximate concentration of the GST-tagged protein, a standard curve may be generated using purified GST.

**Note 2:** If the plate is being used to analyze column effluent samples during the purification of GST proteins, apply samples of each fraction to separate wells and follow steps 3-13 above.

#### References

1. 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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3. 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**, 8972-8976.
4. Frangioni, J V. and Neel, B.G. (1993). Solubilization and Purification of Enzymatically Active Glutathione S-Transferase (pGEX) Fusion Proteins. *Anal. Biochem.* **210**, 179-187.
5. Simons, P. C. and VanderJagt, D.L. (1977). Purification of Glutathione S-Transferases for Human Liver by Glutathione-Affinity Chromatography. *Anal. Biochem.* **82**, 334-341.

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