Express Capture Glutathione Coated Microplates

A rapid screening, enzyme assay, and protein purification tool from XpressBio

Catalog Numbers:

EX7000-C96 (Clear 96 well plate)
EX7000-B96 (Black 96 well plate)
EX7000-W96 (White 96 well plate)
EX7000-C384 (Clear 384 well plate)
EX7000-B384 (Black 384 well plate)
EX7000-W384 (White 384 well plate)

Product Description

Express Capture Glutathione Microplates provide a platform for the capture, purification, and enzymatic assay of glutathione-S-transferase-tagged (GST-tagged) recombinant proteins that is reliable, fast, and has high throughput capabilities. The use of the epitope tag glutathione-S-transferase (GST) is based on the strong affinity of GST for immobilized glutathione-covered matrices. Glutathione-S-transferases are a family of multifunctional cytosolic proteins that are present in eukaryotic organisms. GST isoforms are not usually found in bacteria; thus, endogenous bacterial proteins do not compete with the GST-fusion proteins for binding. This product may be used with GST-tagged proteins for several uses including recombinant protein expression screening, immunoabsorbtion assays, biochemical assays, competition assays and protein purification.

Components

One glutathione coated microplate stored in a foil pouch with desiccant. All catalog numbers above are a solid plate with 96- or 384-wells, with the exception of the clear 96-well plate **(**C96), which is configured in twelve 8-well strips. All plates should be stored at 4° C, and unused plates or strips (C96) should be stored resealed in the foil pouch. The product is stable for one year when stored properly.

Materials and Equipment Required But Not Provided

GST-tagged proteins
Blocking buffers (prepare fresh)
Antibody to recombinant protein
Detection enzyme-conjugate
Enzyme substrate
Microtiter plate reader

Procedures

I. Coupling of GST-tagged Proteins

Buffers:
PBS: Phosphate Buffered Saline, pH 7.4, 10 mM Sodium Phosphate, 2 mM Potassium Phosphate, 135 mM Sodium Chloride, 2.7 mM Potassium Chloride.
PBS Tween (PBST): To PBS add 0.05% (vol/vol) Tween-20.
Blocking Buffers: 1. Dissolve casein (purified powder from Sigma C-5890) to 2 gm/L (0.2%) in PBS-T. Heat to 37° C and stir until dissolved; or 2. Dissolve probumin (Millipore, Catalog# 82-045-1) or BSA to 5 gm/L (0.5%) in PBS-T. Heat to 37° C and stir until the albumin is dissolved.

Recommended Concentrations:

GST-tagged proteins: Purified or partially purified GST-tagged proteins at 1 ng/ml to crude bacterial lysates at 10 mg/ml with 100 µl added per well.

Protocol:

1. Using a wash bottle add 300 µl of PBST buffer to each well. Aspirate or shake out and turn plate upside down and blot on paper towels to remove all liquid. Repeat the wash procedure two times (for a total of three washes).
2. Prepare the GST-tagged protein in PBST or block buffer.
3. Add 100 µl of the protein solution to a well in the microtiter plate.
4. Incubate for one hour at room temperature. Agitation on a rotary mixer can be used (100 rpm).
5. Remove the protein solution and wash using the wash protocol from step 1 with PBST buffer, repeat this wash step a total of 5 times.

II. Detection of GST-tagged Protein

Several methods can be utilized to detect the captured GST-tagged protein. It will usually be

detected with an antibody specific for the protein attached to GST, followed by a secondary antibody that will attach to the primary antibody, and that is conjugated to an enzyme, such as peroxidase. The peroxidase activity gives a time dependent increase in color at a specific wavelength of light that can be detected with a microplate reader. However, direct biochemical assays specific for the protein of interest can also be used. The following example illustrates the detection of recombinant GST-tagged phospholipase C (PLC) protein with primary antibody, a rabbit anti-PLC anti-serum, followed by a secondary biotin-labeled goat anti-rabbit antibody followed by strepavidin conjugated with peroxidase. The relative activity of bound GST-tagged PLC protein is quantitative using ABTS peroxidase substrate and measuring light absorbance at $OD_{405\ nM}$ with a microplate reader.

1. Make an appropriate dilution (1/5 to1/200) of the primary antibody, rabbit anti-PLC antibody, in PBST or in a blocking buffer to reduce background. Add 100 μl of each dilution to a microtiter plate well that has been coupled with the GST-tagged PLC protein (see above).
2. Incubate at room temperature for 60 minutes.
3. Aspirate the wells and tap on paper towels, repeat five times washing with PBST buffer.
4. Optional Blocking Step. In cases in which the primary antibody (polyclonal, old sample, ect.) causes high background in the assay, the anti-serum should be centrifuged to pellet insoluble particles and/or can be pre-absorbed against a parental bacteria lysate (2 μg/μl) for 60 minutes before dilution. Another method to reduce background is to include a blocking step after washing away the GST-tagged protein sample with PBST. Add 100 μl of a blocking buffer to each well and incubate for 45 minutes at room temperature. Aspirate the wells and wash five times with PBST, blotting between washes.
5. Add 100 μl of biotin-labeled goat anti-rabbit antibody (1/5,000) and incubate at room temperature for 60 minutes.
6. Aspirate the wells and wash five times with PBST buffer.
7. Add 100 μl of strepavidin conjugated with peroxidase (1/15000) to each well and incubate at room temperature for 60 minutes.
8. Aspirate the wells and wash five times with PBS-T buffer.
9. Add 100 μl of ABTS peroxidase substrate solution and incubate at room temperature for 20 to 40 minutes. Read the plate on a microplate reader set to $OD_{405\ nM}$.

III. Expression of GST-tagged Protein

Any general protocol for recombinant GST fusion protein expression can be used.
1. Grow bacteria transformed with your GST expression plasmid at 30° C in LB medium containing 100 μg/ml ampicillin.
2. Induce protein expression by adding 0.25 mM of isopropyl-β-D-thio-galactoside (IPTG) to the culture at an $OD_{600\ nM}$ of 0.5 to 0.8.
3. After 4-6 hours of IPTG induction, centrifuge the culture at 6000 xg for 10 minutes.
4. Suspend the bacterial pellet in PBST containing 2 mM DTT and protease inhibitors. Lyse the bacteria with ultrasound. Triton X-100 can be added to 1%.
5. Centrifuge the lysate at 30000 xg at 4° C for 30 minutes and store the sample at -20° C.

Critical Steps

1. Insufficient or excessive washing will result in assay variation and will affect validity of results. Complete removal of the wash buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.
2. High backgrounds can occur from inadequate washing or from too highly concentrated detection materials. Careful titration of ligands will result in optimal assay.
3. Low signals can also occur from inadequate titration of detection materials.
4. All reagents must be at room temperature before running the assay.
5. The optimal dilution of primary antibodies is critical to control background and should be determined experimentally.
6. If high background occurs dilute antibodies in blocking buffer and/or block the well after GST-protein is applied to block against adsorption of secondary antibodies and detection enzymes. Antibody can also be pre-absorbed with a parental bacteria lysate that did not express the protein of interest.

This Product is for Research Use Only. It is not intended for diagnostic or other clinical use

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