FOR RESEARCH USE ONLY!

EZCatch™ GST-Spin Purification Kit

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(Store at 4°C)

Cat. No. K861-10
Contains 10 columns and sufficient reagents to purify a total of ~2.5 mg of recombinant GST-fusion proteins from 10 different samples.

I. Salient Features:

Binding Capacity: Each Spin column can bind upto 250 µg of recombinant protein from 1-5 ml of cell culture or up to 0.25 g of cell pellet.
Column Content: Each Spin column contains 100 µl of high performance Glutathione Sepharose beads (**Cat # 6555**).
Bead Content: A 50% slurry (100 µl of settled resin is equivalent to 200 µl of 50% slurry) in 20% ethanol.

II. Introduction:

The expression and solubility of recombinant proteins can be increased by tagging with Glutathione S-transferase (GST). GST-tag binds to glutathione with high affinity and specificity enabling purification of GST-tagged proteins using glutathione-based affinity resins. BioVision's EZCatch™ GST-Spin Purification Kit is designed for fast, specific and easy purification of such GST-tagged proteins from cell lysates. This kit contains 10 easy-to-use spin columns allowing one-step high quality affinity purification of different GST-tagged proteins. In addition, the kit contains a proprietary Lysis Buffer allowing quick preparation of clear lysates from cell pellets without the need for sonication. Eluted proteins can be directly used for downstream analysis.

III. Applications:

- Rapid purification of GST-fusion proteins from multiple samples
- Optimization of growth conditions for the expression of recombinant proteins
- Pull-down of purified GST-fusion proteins for subsequent Western blot (with GST Antibody – **Cat # 3997**), ELISA and other downstream applications

IV. Kit Contents:

Components	K861-10	Cap Code	Part Number
EZCatch™ GST-Spin Columns	10 columns	-	K861-10-1
EZCatch™ Wash Buffer	5 ml	NM	K861-10-2
EZCatch™ Enzyme Mix	1 vial	Green	K861-10-3
EZCatch™ Lysis Buffer	10 ml	WM	K861-10-4
EZCatch™ Glutathione	1 vial	Yellow	K861-10-5
EZCatch™ Elution Buffer	4 ml	NM/Brown	K861-10-6

V. User Supplied Reagents and Equipment:

- Microcentrifuge, DI Water, centrifuge tubes.

VI. Reagent Preparation and Storage Conditions:

Store the kit at 4°C prior to opening. Read the entire protocol before performing the experiment.

- **EZCatch™ Lysis Buffer and Enzyme Mix:** Equilibrate EZCatch™ Lysis Buffer to room temperature. Briefly spin 1 vial of EZCatch™ Enzyme Mix. Add 0.5 ml of DI water to dissolve its contents and transfer to the EZCatch™ Lysis Buffer. Use the lysis buffer immediately or store at -20°C for long term. Prior to each use, equilibrate the EZCatch™ Lysis Buffer to room temperature.
- **EZCatch™ Elution Buffer:** Add 0.5 ml of the EZCatch™ Elution Buffer to the vial of EZCatch™ Glutathione and resuspend well. Transfer this mixture to the elution buffer bottle and mix well. Use immediately or aliquot and store at -20°C until needed. Prior to use, equilibrate the EZCatch™ Elution Buffer to room temperature.

VII. Protocol for the Purification of GST-tagged Proteins:

Perform all the purification steps at room temperature or at 4°C depending on the stability of your protein.

Cell Lysate preparation:

Grow 1-5 ml of bacterial culture expressing GST-fusion proteins. Pellet the cells and discard supernatant. Resuspend the cell pellet in 1 ml of EZCatch™ Lysis Buffer by gentle vortexing. Incubate the tube at room temperature for about 5-10 min until lysate is clear, vortex gently few times during incubation. Remove cell debris by centrifugation at maximum speed for 10 min at 4°C**.** The supernatant containing the soluble proteins is now ready to be loaded on a EZCatch™ GST-Spin Column.

Column Pre-equilibration:

Equilibrate EZCatch™ GST-Spin column(s) to working temperature. Snap off the bottom plug from the EZCatch™ GST-Spin Column by gentle twisting and save for later use. Centrifuge the column in a microcentrifuge at 700 x g for 2 min to remove the storage buffer **(Centrifugation steps should be carried at a low speed to prevent damage to the resin. Do not vortex the columns)**. Use a 1.5 ml centrifuge tube for collection of the flow-through. Discard the flow-through. Wash the column once with 300 µl of DI water and 300 µl of EZCatch™ Wash Buffer.

Purification:

1. Load 300 µl of the prepared cell lysate on the pre-equilibrated column, close the cap and plug the bottom of the column. Incubate at the desired temperature for 3 min on a rotator or on an end-over-end rocking platform. Remove the plug and spin the column at 700 x g for 2 min, collect the flow-through. Repeat until all the entire cell lysate is passed through the column.
   Notes:
   - If lysis is performed at 4°C, longer incubation times with resin will be needed.
   - If protein is expressed at very low levels, the incubation time can be extended.
   - Do not load more than 300 µl of lysate at once on the GST-Spin Column.
2. Wash the resin at least 3-5 times with 200 µl of EZCatch™ GST Wash Buffer each time. Unplug the column and centrifuge at 700 x g for 2 min. Collect and save all the wash fractions. Monitor the absorbance of the washes at 280 nm ($\mathbf{A_{280}}$) and perform additional washes if necessary until the absorbance approaches baseline.
3. Plug the column and elute the GST-tagged protein from the resin with 100 µl of EZCatch™ Elution Buffer. Incubate the column for 3 min, unplug and centrifuge at 700 x g for 2 min. Repeat this step two more times collecting each eluate in a separate tube. Monitor elution by measuring $\mathbf{A_{280}}$ of the fractions at 280 nm.
4. Analyze the eluted fusion protein by SDS-PAGE, Western blot, ELISA or any other downstream analyses.

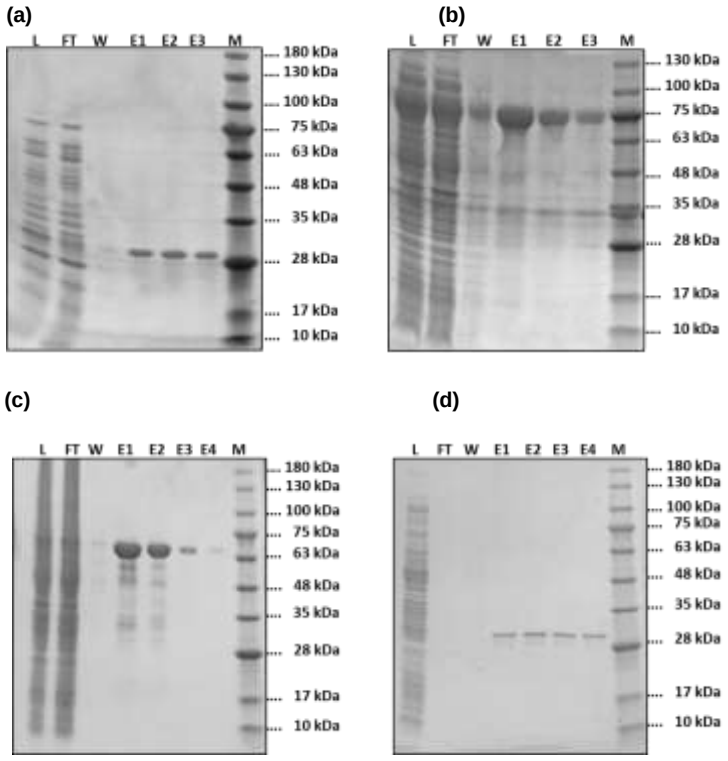

Figure. 4-20 % SDS-PAGE analyses of GST-Fusion proteins purified using EZCatch™ GST-Spin Purification Kit. Samples were purified from the following E.Coli lysates: (a) GST-Δ/GSTD2 protein **(Cat # 7840)** and GST fusion proteins expressed at (b) high, (c) medium, and (d) low levels respectively. The purifications were performed as indicated in the protocol above (M = Protein Marker; L = Crude Lysate; FT = Flow-through; W = Wash, E = Elution)**.**

VIII. RELATED PRODUCTS:

Ni-IDA Spin Columns (6567-25)
Hi-Bind™ Ni QR Agarose Beads (6562)
Benzonase (Cat. #7680)
10K Spin Column (1997)
Ready-to-use Ni QR Agarose Beads Buffer Kit (K6563-3)
Protein G-Sepharose Column (6518)
Protein A/G-Sepharose Column (6528)
Hi-Bind™ Protein A-Agarose (6520)

GST Antibody (3997)
Ready-to-use Ni QR Agarose Beads Buffer Kit (6563-3)
EZ-Desalt™ Spin Desalting Columns (6564-25)
Glutathione Sepharose (6555)
Protein A-Sepharose Column (6508)
Protein L-Sepharose Column (6538)
Protein A/G/L-Sepharose Column (6548)

FOR*: *RESEARCH USE ONLY! Not to be used on humans.