Technical Bulletin

EZview™ Red Glutathione Affinity Gel

E6406

Product Description

EZview™ Red Glutathione Affinity Gel is a highly visible, red-colored glutathione-agarose affinity gel, designed for use in small-scale affinity capture (pull-down) experiments. The affinity resin is composed of glutathione that is covalently attached through its sulfur to epoxy-activated 4% crosslinked agarose beads, to give a 12-atom spacer. This resin is highly selective for proteins with glutathione-binding sequences, such as glutathione S-transferase, glutathione peroxidase, and glyoxalase I.

EZview™ Red Glutathione Affinity Gel captures glutathione-binding target proteins from cell lysates and other biological samples in the same manner as the standard non-colored Glutathione-Agarose (Cat. No. G4510). The glutathione-binding target proteins that bind to the gel are recovered by centrifugation. The red color enhances visibility of the affinity gel to aid in downstream manipulations, such as repetitive washing, and in recovery of the target proteins bound to the affinity resin.

Several publications[4-9] and theses[10] have cited use of this E6406 product in their research protocols.

Reagent

The EZview™ Red Glutathione Affinity Gel is supplied as an ~50% slurry suspension in phosphate buffered saline (PBS), pH 7.2, containing 50% (v/v) glycerol and 15 ppm of Kathon® CG/IPCII, an antimicrobial preservative.

Ligand Density: 10-20 µmoles of glutathione per mL of packed gel volume.

EZview™ products and their use are covered under U.S. Patent numbers 6,887,377, 7,163,633, and 7,438,806.

Equipment Required but Not Provided

Suggested Cat. Nos. are provided as appropriate.

- Appropriate lysis buffer for preparation of cell lysate, such as CelLytic™ M (Cat. No. C2978) or CelLytic™ MT (Cat. No. C3228)
- Vortex mixer
- Protease Inhibitor Cocktail, such as Cat. Nos. P8340 or P2714
- Pipette tips (200 µL)
- Pipette tips, wide orifice (200 µL)
- Pipette tips (1,000 µL)
- Pipette (200 µL)
- Pipette (1,000 µL)
- Microcentrifuge tubes (such as Cat. No. T9661)
- 2× Laemmli Sample Buffer (Cat. No. S3401)
- L-Glutathione, reduced (Cat. No. G4251)

Storage/Stability

EZview™ Red Glutathione Affinity Gel is stable for at least one year when stored at 2-8 °C. Since this product is a slurry containing 50% glycerol, it is considered to be freezer-safe. For maximum stability, it is recommended to store this product at 0 °C to -20 °C. **Do not freeze without 50% (v/v) glycerol present in the storage buffer.**

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Procedure

There are many different procedures and variations for performing small-scale affinity capture (pull-down) experiments. The investigator should choose the specific procedure to suit the particular experiment. The following procedure is a generic method for affinity capture and may not be appropriate for all situations. The procedure is written for a single sample and is appropriate for most mammalian tissue culture cell lines. It can be easily scaled for multiple samples as appropriate. The investigator needs to determine optimal incubation times and conditions.[1]

- The appropriate Lysis Buffer will depend on the organism, type of cells, and experimental objectives. For most affinity capture experiments from mammalian samples, either CelLytic™ M (Cat. No. C2978) or CelLytic™ MT (Cat. No. C3228) is recommended for lysis of mammalian cells and tissues, respectively.
- Manipulations should be carried out on ice or at 2-8 °C.

1. To dispense the affinity gel, carefully mix EZview™ Red Glutathione Affinity Gel beads until uniformly suspended. Add 20-50 µL of the 50% slurry to a clean 1.5 mL microcentrifuge tube on ice. To dispense the beads, use a wide orifice pipette tip or cut ~1 mm off the tip of a regular pipette tip, to enlarge the opening and allow unrestricted flow of the bead suspension. For multiple samples, mix the affinity gel slurry well, before each aliquot, to ensure uniformity of samples.
2. Equilibrate beads in Lysis Buffer by adding 750 µL of the Lysis Buffer to the tube, vortex, and centrifuge in a microcentrifuge for ~30 seconds at 8,200 × *g* (10,000 rpm in an Eppendorf® 5415C microcentrifuge). Carefully remove the supernatant with a micropipette (or carefully aspirate supernatant). Equilibrate the beads a second time. Remove the supernatant. Set the equilibrated red pellet on ice.
3. Prepare the cell lysate using ice cold Lysis Buffer. For most mammalian cells, 0.5-5 × 10^7 cells can be easily lysed in 1 mL of Lysis Buffer. The appropriate protease inhibitor cocktail may be added to the Lysis Buffer, if desired. Transfer the lysate to a 1.5 mL microcentrifuge tube.
4. Immediately centrifuge the lysate for 10 minutes at 8,200 × *g* in a microcentrifuge at 2-8 °C to pellet cell debris.
5. Carefully remove all the clear lysate supernatant from Step 4 with a 1 mL micropipette. Transfer the supernatant into the tube of equilibrated EZview™ Red Glutathione Affinity Gel beads from Step 2. Vortex briefly. Incubate with thorough, gentle mixing for 1 hour at 2-8 °C to allow glutathione-binding proteins to bind to the glutathione on the affinity gel.
6. Centrifuge in microcentrifuge for 30 seconds at 8,200 × *g*. Set on ice. Aspirate supernatant carefully (or remove with a micropipette). Set tube with the bead pellet on ice.
7. Wash bead pellet by adding 750 µL of Lysis Buffer. Vortex briefly. Incubate with thorough, gentle mixing at 2-8 °C for 5 minutes. Centrifuge in microcentrifuge for 30 seconds at 8,200 × *g*. Aspirate supernatant carefully (or remove with a micropipette). Set tube with the bead pellet on ice.
8. Repeat washes two more times as in Step 7. Remove the final wash supernatant. Elute the bound protein from the bead pellet. Analyze as desired (see Analysis of Bound Protein).

Note: Controls may be useful in pull-down experiments. Perform incubations with non-relevant protein, or do a competition study with free, reduced glutathione to determine binding specificity. Include a control sample without the glutathione-binding protein to determine non-specific protein binding, if desired.

Analysis of Bound Protein

Elution of bound protein from the gel with glutathione

The glutathione-binding protein may be eluted from the resin with reduced glutathione (Cat. No. G4251):

- Add the desired volume of freshly prepared 5-10 mM reduced glutathione in 50 mM Tris-HCl (pH 7.5).
- Incubate with the affinity gel sample for 5 minutes.
- Recover the supernatant after pelleting the affinity gel by centrifugation.

SDS-PAGE analysis

To elute the captured protein from the bead pellet for SDS-PAGE analysis:

- Add 25 µL of Lysis Buffer to the tube. Vortex briefly.
- Then add 25 µL of 2× Laemmli sample buffer. Vortex briefly.
- Boil sample for 5 minutes. Vortex.
- Centrifuge 30 seconds at 8,200 × *g* in a microcentrifuge to pellet the EZview™ Red Glutathione Affinity Gel beads.
- Retrieve the supernatant. Store the supernatant frozen, if not using it immediately.
- Run 10-20 µL of the supernatant on a denaturing SDS-PAGE gel.
- Perform subsequent detection by staining, autoradiography, or immunoblotting, as desired.

Note: For analysis using non-reducing SDS-PAGE, use a sample buffer without reducing agents such as 2-mercaptoethanol or dithiothreitol.

Enzyme assays

- Enzyme assays, such as kinase assays, can be performed by adding the assay mixture and substrate directly into the bead sample tube.
- The bead pellet should first be equilibrated in the assay buffer, by pre-washing in assay buffer before the assay, similar to Step 2, except that the enzyme assay buffer is used in place of Lysis Buffer.

References

1. Simons, P.C., and Vander Jagt, D.L., *Analytical Biochem.*, **82(2)**, 334-341 (1977).
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4. Guo, C. *et al.*, *Curr. Biol.*, **17(8)**, 700-705 (2007).
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6. Kosako, H. *et al.*, *Nat. Struct. Mol. Biol.*, **16(10)**, 1026-1035 (2009).
7. Woo, C.W. *et al.*, *Nat. Cell Biol.*, **14(2)**, 192-200 (2012).
8. Kweon, S.-M. *et al.*, *Cell Rep.*, **21(2)**, 482-494 (2017).
9. Wang, H. *et al.*, *Nat. Commun.*, **9(1)**, 3683 (2018).
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Related Products

- Protease inhibitor cocktails (for general, bacterial, mammalian, fungal & yeast, plant, tissue culture, and histidine-tagged) (Cat. Nos. P2714, P8465, P8340, P8215, P9599, P1860, and P8849)
- Phosphatase inhibitor cocktails (Cat. Nos. P2850 and P5726)
- BCA protein assay kits:
  - Standard Protein Determination Kit (Cat. No. BCA1)
  - QuantiPro™ BCA Assay Kit (Cat. No. QPBCA)
- EZBlue™ Gel Staining Reagent (Cat. No. G1041)
- Glutathione, reduced (Cat. No. G4251)
- Glutathione-agarose (Cat. No. G4510)
- EZview Red Protein A Affinity Gel (Cat. No. P6486)
- EZview Red Protein G Affinity Gel (Cat. No. E3403)
- EZview Red ANTI-FLAG® M2 Affinity Gel (Cat. No. F2426)
- EZview Red HIS-Select® HC Nickel Affinity Gel (Cat. No. E3528)

Notice

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Troubleshooting Guide

The enhanced visibility of the red affinity resin beads makes it is easy to see if the beads have been accidentally removed during the wash steps. If this happens, simply return the wash supernatant to the tube, and repeat the centrifugation step to pellet the resin again.

Problem	Possible Cause	Solution
No signal is observed.	Glutathione-binding protein is not present in the sample.	• Verify that the protein of interest contains the glutathione-binding protein by immunoblot or dot blot analyses. • Prepare fresh lysates. Avoid using frozen lysates. • Use appropriate protease inhibitors in the lysate. Alternatively, increase their concentrations to prevent degradation of the glutathione-binding protein.
	Washes are too stringent.	• Reduce the number of washes. • Avoid adding high concentrations of NaCl to the mixture. • Use solutions that contain less or no detergent.
	Incubation times are inadequate.	Increase the incubation times with the affinity resin (from several hours to overnight).
	Interfering substance is present in sample.	• Lysates containing high concentrations of oxidizing agents or high concentrations dithiothreitol (DTT), 2-mercaptoethanol, or other reducing agents may destroy protein structure and function, and must be avoided. • Excessive detergent concentrations may also interfere with the protein binding interactions.
	Detection system is inadequate.	If Western blotting detection is used: • Check primary and secondary antibodies using proper controls to confirm binding and reactivity. • Verify that the transfer was adequate by staining the membrane with Ponceau S. • Use fresh detection substrate. Alternatively, try a different detection system.
Background is too high.	Proteins bind non-specifically to the resin beads or the microcentrifuge tubes.	• Pre-clear lysate with 4% crosslinked agarose beads. • After suspending beads for the final wash, transfer the entire sample to a clean microcentrifuge tube before centrifugation.
	Washes are insufficient.	• Increase the number of washes. • Prolong the duration of the washes, incubating each wash for at least 15 minutes. • Increase the salt and/or detergent concentrations in the wash solutions. • Centrifuge at lower speed to avoid non-specific trapping of denatured proteins from the lysate during the initial centrifugation of the affinity resin complexes.

Reagent Compatibility Table

Reagent	Comments
Buffers	Tris or phosphate buffers, pH 6.5 to 9.5, are compatible with affinity capture with glutathione-agarose.
Salt	Salt concentrations up to 1 M do not interfere with specific binding to the glutathione-agarose.
Protease inhibitors	Protease inhibitors such as EDTA, PMSF, or other serine protease inhibitors do not interfere with binding.
Detergents	The binding of GST to glutathione-agarose is unaffected by 1% TRITON™ X-100, 1% TWEEN® 20, 1% CTAB, or 0.03% SDS.
Reducing agents	The binding of GST to glutathione-agarose is unaffected by 10 mM DTT.