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Product Information

EZview™ Red Protein G Affinity Gel

Catalog Number **E3403**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

EZview™ Red Protein G Affinity Gel is a highly visible, red colored Protein G affinity gel, designed for use in immunoprecipitation experiments. The affinity resin contains Protein G′, a truncated, recombinant form of the Type G *Streptococci* cell wall protein, covalently attached to crosslinked 4% agarose beads. The recombinant Protein G retains the two Fc binding sites, but lacks the albumin binding sites of native Protein G.

EZview Red Protein G Affinity Gel functions to bind antibody (IgG) at the Fc portion of the antibody in the same manner as the standard non-colored Protein G Affinity Gel, in order to purify antibody-antigen complexes from cell lysates and other biological samples. The immune complexes, bound to the EZview Red Protein G Affinity Gel, are recovered by centrifugation.

The red color gives the affinity gel enhanced visibility in order to aid in manipulations, such as repetitive washings, and recovery of the target proteins bound to the affinity resin. The enhanced visibility results in less tedious sample processing and greater experimental reproducibility for more accurate quantitation of the proteins of interest.

EZview Red Protein G Affinity Gel is supplied as an ~50% slurry suspension in phosphate buffered saline (PBS) containing 50% glycerol and 0.0015% (15 ppm) Kathon® CG/IPCII, as an antimicrobial preservative. Binding capacity for rabbit IgG is ~8 mg/ml of packed gel.

Equipment and Reagents to be Supplied by User

Catalog Numbers are provided as appropriate.

- Cells to be used for preparation of lysate
- Appropriate lysis buffer such as RIPA buffer (R0278), CelLytic™ M (C2978), or CelLytic MT (C3228), CelLytic B (B7435, B7310, or C8740), CelLytic P (C2360), or CelLytic Y (C2360)
- Vortex Mixer
- Protease Inhibitor Cocktail (P8340)
- Pipette tips (200 µl) (P5161)
- Pipette tips, wide orifice (200 µl) (P1678)
- Pipette tips (1000 µl) (P1665)
- Pipette tips (1000 µl) (P1665)
- Pipette (200 µl) (Z368113)
- Pipette (1000 µl) (Z368121)
- Microcentrifuge Tubes (T9661)
- 2× Laemmli Sample Buffer (S3401)

Precautions and Disclaimer

EZview Red Protein G Affinity Gel is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

EZview Red Protein G Affinity Gel should be stored in 50% glycerol at –20 °C. This product is stable for at least one year when stored as indicated. Do not freeze in the absence of glycerol.

Procedure

Note: There are many different procedures and variations for performing immunoprecipitation experiments. The specific method should be chosen by the investigator to suit the particular experiment. See Reference 1 for additional information and procedures.

The following generic procedure is intended to be an example or starting point and may not be appropriate for all situations. The procedure is written for one sample and is appropriate for most mammalian tissue culture cell lines. It can be easily scaled for more samples, as appropriate. Optimal incubation times and conditions should be determined by the investigator.

Manipulations should be carried out on ice or at 2–8 °C.

Immunoprecipitation procedure

1. Prepare cell lysate using ice-cold RIPA buffer (R0278) or other suitable lysis buffer. For most mammalian cells, 0.5–5 × 10^7 cells can be easily lysed in 1 ml of RIPA lysis buffer. Include an appropriate protease inhibitor cocktail if desired. Transfer lysate to a 1.5 ml microcentrifuge tube.
2. Immediately centrifuge lysate for 10 minutes at 8,200 × *g* in a microcentrifuge (e.g., 10,000 rpm in an Eppendorf® 5415C microcentrifuge) at 2–8 °C to pellet cell debris. Carefully transfer clear supernatant into a clean microcentrifuge tube on ice.
3. Add 1–10 µl of an appropriate dilution of antibody to 0.1–1.0 ml of lysate sample. The amount of antibody to add may be determined from the antibody product data specifications or may be determined by standard titration methods. Vortex briefly and incubate with thorough, gentle mixing for 1 hour at 2–8 °C to allow antibody-antigen complexes to form.
4. Carefully mix EZview Red Protein G Affinity Gel beads until completely and uniformly suspended. Aliquot 20–50 µl of the 50% slurry into a clean 1.5 ml microcentrifuge tube on ice. To dispense 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.
5. Wash/equilibrate beads in lysis buffer: Add 750 µl of lysis buffer to the tube, vortex, and centrifuge in a microcentrifuge for ~30 seconds at 8,200 × *g*. Carefully remove the supernatant with a pipette (or carefully aspirate supernatant) and set tube with the bead pellet on ice.
6. Repeat wash as above. After removing supernatant, set washed bead pellet on ice.
7. Briefly centrifuge the tube of lysate plus antibody from step 3 for several seconds at 8,200 × *g* to collect all the liquid to the bottom part of the microcentrifuge tube. Carefully remove all of the lysate with a 1 ml pipette and transfer into the tube of washed EZview Red Protein G Affinity Gel beads from step 6. Vortex briefly and incubate with thorough, gentle mixing for 1 hour at 2–8 °C to allow antibody-antigen complexes to bind to the Protein G on the EZview Red Protein G Affinity Gel beads.
8. Centrifuge in a microcentrifuge for 30 seconds at 8,200 × *g*. Set on ice. Aspirate supernatant carefully (or remove with a pipette) and set tube with the bead pellet on ice.
9. Wash bead pellet by adding 750 µl of lysis buffer. Vortex briefly and 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 pipette) and set tube with the bead pellet on ice.
10. Repeat washes two more times as in step 9. After removing the final wash supernatant, the bound antigen can be eluted from the bead pellet and analyzed as desired (see Analysis of Results).

Analysis of Results

SDS-PAGE analysis

To elute antibody-antigen complexes from the bead pellet for SDS-PAGE analysis, add 25 µl of lysis buffer to each tube, vortex briefly, then add 25 µl of 2× sample buffer. Vortex briefly. Boil samples for 5 minutes, vortex and centrifuge for 30 seconds at 8,200 × *g* in a microcentrifuge to pellet the EZview Red Protein G Affinity Gel beads. Freeze eluted bead samples for storage, if not used immediately. Run 10–20 µl of supernatant on a denaturing SDS/PAGE gel. Perform subsequent analysis 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 assay mixture and substrate directly into the bead sample tube. The bead pellet first should be equilibrated in assay buffer by pre-washing in assay buffer before the assay.

Controls

Perform incubations with non-relevant antibody or do blocking with specific peptide antigen(s) as specificity controls. Include a control sample without antibody to determine non-specific protein binding, if desired.

Troubleshooting Guide

Because of the enhanced visibility of the affinity resin beads, it is easy to see if beads are accidentally removed during wash steps. If this happens, simply put the wash supernatant back into the tube and repeat the centrifugation step to pellet the resin again.

Problem	Possible Cause	Solution
No signal is observed.	Specific antibody is not capable of immunoprecipitation.	• Try a different antibody. Not every antibody, especially a monoclonal antibody, can immunoprecipitate. • Add a bridging antibody (from a class or species with higher affinity for Protein G) which is reactive with the primary antibody.
	Antigen of interest is not present in the sample.	• Verify that the sample contains the antigen of interest by immunoblot or dot blot analyses. • Prepare fresh lysates. Avoid using frozen lysates. • Use appropriate protease inhibitors in sample or increase their concentration to prevent degradation of protein antigens.
	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 dithiothreitol (DTT), 2-mercapto-ethanol, or other reducing agents may destroy antibody function and must be avoided. • Excessive detergent concentrations can interfere with the protein binding interactions. Try diluting the lysate with buffer prior to immunoprecipitation.
	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 solution. • Use fresh detection substrate or try a different detection system.
Background is too high.	Proteins bind non-specifically to Protein G, the resin beads, or the microcentrifuge tube.	• Pre-clear the sample once or several times before adding antibody by preincubation with EZview Red Protein G Affinity gel to remove non-specific binding proteins. • 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. • Increase the duration of the washes, incubating each wash for at least 15 minutes. • Increase the salt and/or detergent concentrations in the washing solutions. • Centrifuge at lower speed to avoid non-specific trapping of lysate proteins during the initial centrifugation of the Protein G/antigen complexes.

Protein G - IgG Binding Table

Species	Immunoglobulin	Protein G Binding
Human	IgG (normal)	++++
	IgG1	++++
	IgG2	++++
	IgG3	++++
	IgG4	++++
	IgM	—
	IgA	—
	IgE	—
	IgD	—
	Fab	++
	K light chains	—
	L light chains	—
	ScFv	—
Mouse	IgG1	++++
	IgG2a	++++
	IgG2b	+++
	IgG3	+++
Rat	IgG1	+
	IgG2a	++++
	IgG2b	++
	IgG2c	++
Bovine	IgG	++++
Cat	IgG	—
Chicken	IgG	+
Dog	IgG	++++
Goat	IgG	++
Guinea Pig	IgG	++
Hamster	IgG	++
Horse	IgG	++++
Pig	IgG	+++
Rabbit	IgG	+++
Sheep	IgG	++

References

1. Harlow, E. and Lane, D., Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York, p. 423-470 (1988).
2. Kronvall, G., A surface component in group A, C and G streptococci with non-immune reactivity for immunoglobin G. *J. Immunol.*, **111,** 1401-1406 (1973).
3. Björck, L., and Kornvall G., Purification and some properties of streptococcal protein G, a novel IgG-binding reagent. *J. Immunol.* **133,** 969-974 (1984).
4. Äkerström, B., Protein G: a powerful tool for binding and detection of monoclonal antibodies. *J. Immunol.* **135,** 2589-2592 (1984).
5. Äkerström, B., and Björck, L., A physicochemical study of protein G, a molecule with unique immunoglobulin G-binding properties. *J. Biol. Chem.*, **261,** 10240-10274 (1986).

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