Technical Bulletin

EZview™ Red Anti-HA Affinity Gel

E6779

Product Description

Human influenza hemagglutinin (HA) is a surface glycoprotein that is required for infectivity of the human virus.[1] Many recombinant proteins have been engineered to express a short tag sequence that corresponds to amino acids 98-106 from the HA protein. This HA-tag facilitates the detection, isolation, and purification of such tagged proteins.[2-6]

EZview™ Red Anti-HA Affinity Gel is a highly visible, red-colored anti-HA agarose affinity gel, designed for use in immunoprecipitation (IP) experiments. The affinity resin contains anti-HA monoclonal antibody (affinity-purified from ascites fluid of hybridoma HA-7, using Protein A agarose) which is covalently attached to crosslinked agarose beaded particles at 2.0-2.4 mg of antibody per mL of packed gel.

EZview™ Red Anti-HA Affinity Gel functions to bind HA-tagged proteins from cell lysates and other biological samples in the same manner as non-colored Monoclonal Anti-HA Agarose Conjugate (Cat. No. A2095). The HA-tagged proteins, bound to the Anti-HA Affinity Gel, are recovered by centrifugation. This product is designed for IP of HA-tagged fusion proteins from lysates of bacterial or mammalian cells or other biological samples.

The red color gives this affinity gel enhanced visibility, to facilitate procedural steps such as repetitive washings, recovery of the affinity resin beads, and recovery of bound HA-tagged proteins. Several theses[7-8] and dissertations[9-17] have cited use of this E6779 product in their research protocols.

Reagent

EZview™ Red Anti-HA Affinity Gel is supplied as a ~50% slurry suspension in phosphate buffered saline (PBS) with 50% glycerol and 0.0015% (15 ppm) Kathon® CG/IPCII, as an antimicrobial preservative.

Binding Specificity: HA-tag peptide (N-Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala-C). EZview™ Red Anti-HA Affinity Gel recognizes native as well as denatured or reduced forms of HA-tagged proteins. It detects N-terminal or C-terminal HA-tagged fusion proteins.

Binding capacity: for a purified, HA-tagged 27 kDa fusion protein, ~0.4 mg/mL of packed resin

Equipment Required but Not Provided

Suggested Cat. Nos. are provided as appropriate.

- Appropriate lysis buffer, such as CelLytic™ M (Cat. No. C2978), CelLytic™ MT (Cat. No. C3228), or RIPA Buffer (Cat. No. R0278)
- 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)

Storage/Stability

EZview™ Red Anti-HA 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 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

Various IP procedures are available.[18] Investigators should choose the specific method to suit the particular experiment.

The following procedure is a generic method for affinity capture of HA-tagged proteins for protein-protein interaction studies. It 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. Investigators need to determine optimal incubation times and conditions. Manipulations should be done on ice or at 2-8 °C.

The Lysis Buffer will depend on the organism, type of cells, and experimental objectives. RIPA buffer (Cat. No. R0278), CelLytic™ M (Cat. No. C2978), or CelLytic™ MT (Cat. No. C3228) may be used for lysis of most mammalian cells or tissues.

Immunoprecipitation of HA-tagged proteins

1. Carefully mix EZview™ Red Anti-HA Affinity Gel beads until completely and uniformly suspended. Add 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.
2. Equilibrate beads in Lysis Buffer by adding 750 µL of Lysis Buffer to the tube, vortex, and centrifuge in a microcentrifuge for approximately 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). Set the tube with the bead pellet on ice. Equilibrate the beads a second time. After removing the supernatant, set the tube with equilibrated red bead 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 the clear lysate supernatant from Step 4 with a 1 mL micropipette. Transfer into the tube of equilibrated EZview™ Red Anti-HA Affinity Gel beads from Step 2. Vortex briefly. Incubate with thorough, gentle mixing for 1 hour at 2-8 °C to allow HA-tagged proteins to bind to the anti-HA antibody on the EZview™ Red Anti-HA Affinity Gel.
6. Centrifuge in a microcentrifuge for 30 seconds at 8,200 × *g*. Set on ice. Aspirate supernatant carefully (or remove with a micropipette). Set the tube with the bead pellet on ice.
7. Wash the 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 a microcentrifuge for 30 seconds at 8,200 × *g*. Aspirate supernatant carefully (or remove with a micropipette). Set the tube with the bead pellet on ice.
8. Repeat washes two more times as in Step 7. After removing the final wash supernatant, the bound protein can be eluted and analyzed as desired.

Analysis of Bound Protein

Elution of the HA-fusion protein with HA peptide

HA-tagged protein bound to the resin may be eluted with HA peptide (Cat. No. I2149):

- Add the desired volume of a freshly prepared 100 µg/mL solution of HA peptide in RIPA buffer.
- Incubate the affinity gel sample for at least 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 50 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 EZview™ Red Anti-HA Affinity Gel beads.
- Run 10-20 µL of the supernatant on a denaturing SDS-PAGE gel.
- Store sample frozen, if not used immediately.
- Perform subsequent detection by staining, autoradiography, or immunoblotting, as desired.

Note: For analysis by 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 as in Step 2, except using enzyme assay buffer in place of Lysis Buffer.

Troubleshooting Guide

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

Problem	Possible Cause	Solution
No signal is observed	HA-tagged protein is not present in the sample.	• Make sure the protein contains the HA-tag by immunoblot or dot blot analyses. • Prepare fresh lysates. Avoid using frozen lysates. • Use appropriate protease inhibitors in the lysate or increase their concentrations to prevent degradation of the HA-tagged 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 dithiothreitol (DTT), 2-mercaptoethanol, or other reducing agents may destroy antibody 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 with proper controls to confirm binding and reactivity in detection system. • Verify that the transfer was adequate. • Try fresh detection substrate or try a different detection system.
Background is too high.	Proteins non-specifically bind to the anti-HA monoclonal antibody, the resin beads, or the microcentrifuge tubes.	• Pre-clear lysate with Mouse IgG-Agarose (Cat. No. A0919) and/or EZview™ Red Protein A Affinity Gel (Cat. No. P6486) to remove non-specific binding proteins. • After suspending beads for the final wash, transfer entire sample to a clean microcentrifuge tube before centrifugation.
	Washes are insufficient.	• Increase the number of washes • Prolong duration of the washes, incubating each wash for at least 15 minutes. • Increase the salt and/or detergent concentrations in washing 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	Effect	Comments
Chaotropic agents (such as urea, guanidine HCl)	Denatures the immobilized anti-HA antibody	• **Do not** use any reagent that contains chaotropic agents, since chaotropic agents denature the anti-HA antibody on the resin and destroy its ability to bind HA-tagged proteins. • If necessary, low concentrations of urea (1 M or less) can be used.
Reducing agents (such as DTT, DTE, 2-mercaptoethanol)	Reduces the disulfide bridges holding the anti-HA antibody chains together	**Do not** use any reagent that contains reducing agents, since reducing agents will reduce the disulfide linkages in the anti-HA antibody on the resin and destroy its ability to bind the HA-tagged proteins.
Sodium dodecyl sulfate (SDS)	Reduces non-specific protein binding to the resin	May be used up to a concentration of 0.1%. **Do not exceed**, since SDS may denature the anti-HA antibody on the resin and destroy its ability to bind HA-tagged proteins.
TWEEN® 20	Reduces non-specific protein binding to the resin	May be used up to a concentration of 5%. **Do not exceed**.
TRITON® X-100	Reduces non-specific protein binding to the resin	May be used up to a concentration of 5%. **Do not exceed**.
IGEPAL® CA-630 (NP-40)	Reduces non-specific protein binding to the resin	May be used up to a concentration of 1%. **Do not exceed**.
Digitonin	Reduces non-specific protein binding to the resin	May be used up to a concentration of 0.2%. **Do not exceed**.
Sodium chloride	Reduces non-specific protein binding to the resin by reducing ionic interactions	May be used up to a concentration of 1.0 M. **Do not exceed**.
0.1 M glycine HCl, pH 2.5	Elutes HA-tagged protein from the resin	Do not leave the affinity resin in glycine-HCl for longer than 20 minutes. Longer incubation times will begin to denature the anti-HA antibody.

Related Products

- Anti-HA-Peroxidase Conjugate (Cat. No. H6533)
- Anti-HA-Alkaline Phosphatase Conjugate (Cat. No. A5477)
- Anti-HA-FITC Conjugate (Cat. No. H7411)
- Anti-HA-TRITC Conjugate (Cat. No. H9037)
- Anti-HA-Biotin Conjugate (Cat. No. B9183)
- Protease and phosphatase inhibitor cocktails (Cat. Nos. P2714, P8465, P8340, P8215, P9599, P2850, P5726, P0044, MSSAFE, P0001, PIC0002, PIC0004, PIC0005, PIC0006, PPC2020)
- BCA protein assay kits: Standard (Cat. No. BCA1) and QuantiPro™ (Cat. No. QPBCA)
- EZview™ Red Protein A Affinity Gel (Cat. No. P6486)

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