Technical Bulletin

EZview™ Red Streptavidin Affinity Gel

E5529

Product Description

Streptavidin is a 66 kDa homotetrameric protein from *Streptomyces avidinii* that is similar to avidin with respect to its high affinity for biotin ($K_A \sim 10^{15}$ M^{-1}).[1-4] Streptavidin is slightly anionic (pI 5-6) and non-glycosylated. These properties contribute to its relatively low non-specific binding compared to egg white avidin (a glycoprotein with pI ~10.5).

Streptavidin-agarose can be used to immobilize or isolate various biotinylated macromolecules and complexes (proteins, antibodies, lectins, nucleic acids, receptors, and ligands).[5-11] In binding of biotinylated macromolecules to streptavidin, harsh conditions are needed to disrupt the streptavidin-biotin interaction.

EZview™ Red Streptavidin Affinity Gel is a highly visible, red-colored streptavidin agarose affinity gel, designed for use in small-scale affinity capture (pull-down) experiments. The EZview™ Red Streptavidin Affinity Gel is composed of streptavidin that is covalently attached to CNBr-activated 4% agarose beads through an amino group, to give a 7-atom spacer.

EZview™ Red Streptavidin Affinity Gel captures biotinylated target molecules, such as proteins, and interacting molecules from cell lysates and other biological samples in the same manner as the standard non-colored Streptavidin-Agarose (Cat. No. S1638). The biotinylated target proteins that bind to the gel are recovered by centrifugation. The red color gives the affinity gel enhanced visibility, which aids in downstream manipulations, such as repetitive washings, and recovery of the target proteins bound to the affinity resin.

Several publications[12-17] and dissertations[18-21] have cited use of E5529 in their research protocols.

Product

The EZview™ Red Streptavidin 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: 0.8-2 mg of streptavidin per mL of packed gel volume.

Binding capacity: ~10 µg biotin 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 to prepare cell lysate, such as RIPA buffer (Cat. No. R0278), CelLytic™ M (Cat. No. C2978), or CelLytic™ MT (Cat. No. C3228), CelLytic™ B (Cat. Nos. B7435, B7310, or C8740), CelLytic™ P (Cat. No. C2360), or CelLytic™ Y (Cat. No. C2360)
- 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 Streptavidin 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 -20 °C. **Do not freeze in the absence of glycerol.**

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

Many different procedures and variations are available to perform 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 of biotinylated proteins for protein-protein interaction studies, 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.

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

Manipulations should be done on ice or at 2-8 °C.

1. Carefully mix EZview™ Red Streptavidin 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 affinity gel slurry well before each aliquot to ensure uniformity of samples.
2. Equilibrate the beads in Lysis Buffer by adding 750 µL of the Lysis Buffer to the tube. Vortex. Centrifuge in microcentrifuge for ~30 seconds at 8,200 × *g* (such as 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. After removing 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 the Lysis Buffer. An appropriate protease inhibitor cocktail may be added to the Lysis Buffer, if desired. If a translucent, viscous aggregate of denatured DNA is present, carefully remove it with a pipette. 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 and transfer into the tube of equilibrated EZview™ Red Streptavidin Affinity Gel beads from Step 2. Vortex briefly. Incubate with thorough, gentle mixing for 1 hour at 2-8 °C to allow biotinylated proteins to bind to the streptavidin on the EZview™ Red Streptavidin 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 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. After removing the final wash supernatant, the bound protein can be eluted from the bead pellet and analyzed 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 streptavidin to determine binding specificity. Include a control sample lacking the biotinylated protein to determine non-specific protein binding, if desired.

Analysis of Bound Protein

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.
- Add 25 µL of 2× Laemmli sample buffer.
- Vortex briefly.
- Boil sample for 5 minutes.
- Vortex.
- Centrifuge for 30 seconds at 8,200 × *g* in a microcentrifuge, to pellet the EZview™ Red Streptavidin Affinity Gel beads.
- Store frozen, if not used 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.

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 the enzyme assay buffer is used in place of Lysis Buffer.

Immunoaffinity Capture

Specific antibodies can be biotinylated and bound to EZview™ Red Streptavidin Affinity Gel. After binding the antibody and washing the affinity gel, the bound antibody can be used to capture the target antigen protein from a lysate or solution. The target protein can be eluted using relatively mild conditions that leave the biotinylated antibody bound to the streptavidin affinity gel. For example, the target antigen protein can be eluted with 0.1 M acetic acid, 0.1 M glycine HCl, or another buffer, in the range of pH 1.8-2.5, to dissociate the antibody-antigen interaction. After elution at low pH, the samples should be immediately neutralized to help maintain native structure and activity.[22,23]

Note: Relatively harsh and denaturing conditions are needed to disrupt the very strong biotin-streptavidin interaction. Biotinylated proteins may be eluted from the resin and recovered using 6 M guanidine-HCl, pH 1.5-2, or by boiling the resin in 2% SDS with 0.4 M urea. Incubate with the affinity gel sample for 5 minutes, and recover the supernatant after pelleting the affinity gel by centrifugation.

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 put the wash supernatant back into the tube, and repeat the centrifugation step to pellet the resin again.

A possible option to avoid the harsh denaturing conditions necessary to elute biotinylated proteins from the affinity gel is to iminobiotinylate the protein of interest. Iminobiotin has a pH-dependent interaction with streptavidin which allows tight binding at pH >9.5 and dissociation at pH 4.[24,25]

For biotinylated nucleic acid probes, DTT or 2-mercaptoethanol can reportedly be used to disrupt the streptavidin-biotin interaction.[26]

Binding inhibitors: Non-dialyzed, nonfat dried milk, which may contain biotin and various sugars (such as mannose), is reported to interfere with the binding of biotin to streptavidin and avidin.[27,28]

Reduction of non-specific binding:

If non-specific binding is a problem, consider the following steps:[29]

- Use a different buffer system, or increase the concentration of NaCl to 0.5 M.
- Increase detergent concentration, or use a different detergent or combination of detergents.
- Use alternating high and low pH washes to remove non-specifically bound proteins. One example is:
  - High pH buffer wash, such as with borate buffer (pH 8-9),
  - Followed with a low pH buffer, such as acetate buffer (pH 4).

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