DYKDDDDK (FLAG® tag) IP Protocol

Product: Anti-DYKDDDDK (FLAG® tag) Affinity Gel
Catalog Number: 200-350-383
Storage Temperature: 4 °C

I. Product Description

Anti-DYKDDDDK Affinity Gel is a purified mouse IgG2a monoclonal antibody coupled to activated agarose. This product is intended for purification of proteins containing the FLAG® epitope tag sequence.

II. Binding Specificity

Anti-DYKDDDDK Affinity Gel binds the FLAG® epitope tag sequence (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) fused to the amino terminal, carboxy terminal or internal locations of targeted recombinant proteins expressed in transfected or transformed cells. D-Y-K-D-D-D-D-K peptide is recommended for competitive elution to recover fusion protein.

III. Reagent/Storage

Anti-DYKDDDDK Affinity Gel is supplied in 0.02M Potassium Phosphate, 0.15M Sodium Chloride pH 7.2 in 50% (v/v) glycerol and 0.01% (w/v) sodium azide (added as a preservative). Store vial at 4° C prior to opening vial. The unopened product is stable for one year when stored appropriately. After use, anti-DYKDDDDK Affinity Gel should be cleaned and stored in with PBS and azide. Do not freeze in the absence of glycerol.

IV. Intended Use – Please read the entire protocol prior to use

Anti-DYKDDDDK Affinity Gel is optimally suited for immunoprecipitation and purification of FLAG® tagged proteins. Anti-DYKDDDDK Affinity Gel antibody recognizes the FLAG® epitope tag fused to the amino terminal, carboxyl terminal ends or an internal location of targeted fusion proteins. The epitope tag peptide sequence was first derived from the 11-amino-acid leader peptide of the gene-10 product from bacteriophage T7. DYKDDDDK is the most commonly used hydrophilic octapeptide tag. The control peptide D-Y-K-D-D-D-D-K is recommended for competitive elution to recover proteins.

Remove only the amount of gel necessary for the intended purification (binding capacity is >1 µg protein per 10 µL of gel). Swirl the vial to thoroughly resuspend the gel, remove the desired amount, and transfer the gel to a microcentrifuge tube.

V. Purification

a. Clarify the extract containing the FLAG® tagged protein obtained from cell lysates (provided by user) to remove any insoluble material. Typically insoluble material is removed by centrifugation at 12,000 rpm for 15-20 minutes, or other similar methods. Filter the protein extract using a 0.22 µm or 0.45 µm filter to remove any remaining particulate that otherwise may obstruct the flow of buffer through the gel in later steps.

Transfer the protein extract to the microcentrifuge tube containing the Anti-DYKDDDDK Affinity Gel and incubate for at least 3 hours at 4 °C with gentle mixing to capture the FLAG® tagged proteins. Mixing should be done using a rocking platform. Do not use a magnetic stirring system because this will destroy the gel beads. Some proteins may require overnight incubations to maximize the opportunity for antibody to bind. In these instances it may be necessary to add protease inhibitors and/or bacteriostatic agents (provided by user) to prevent proteolysis and microbial growth.

b. Once the binding step is complete, collect the affinity gel from the microcentrifuge tube by centrifugation (2000-3000 rpm for 5 minutes) or by filtration using an appropriately sized apparatus. Wash the affinity gel with 10 gel volumes of Wash Buffer (100 mM Tris-HCl, 150 mM NaCl, pH 7.5 - 10X TBS pH 7.5) to remove binding of nonspecific proteins. Check the last wash by UV absorbance to confirm that no protein is present. Repeat washing if needed. After the last centrifugation aspirate the Wash Buffer from the affinity gel.

c. Elute FLAG® tagged protein from the affinity gel by peptide competition using the control FLAG® peptide D-Y-K-D-D-D-D-K (purchased separately). Add 3 gel volumes of control peptide to the affinity gel prepared from a stock concentration to a final concentration of 0.5 mg/mL in Wash Buffer. Incubate the mixture for 30 minutes at 4 °C with mixing as before. Centrifuge to collect the affinity gel and recover the supernatant which contains the eluted FLAG® tagged protein. The amount of FLAG® tagged protein present can be monitored by measuring the absorbance of the supernate at 280 nm [if the extinction coefficient for the protein is known]. Elution conditions using Triple FLAG® peptide must be optimized by the user.

d. Larger scale purification may be optimized for column chromatography. In these instances protease inhibitors and/or bacteriostatic agents should be added to Wash Buffer.

e. Conditions for using SDS-PAGE Sample Buffer, 0.1 M Glycine, pH 3.5, or other high pH or low pH elution buffers to elute FLAG® tagged protein from the affinity gel must be optimized by the user. Exposure to harsh conditions may inactivate the ability of the antibody to bind protein thereby inactivating the gel for reuse.

f. Do not use the following buffers:

- Chaotropic reagents such as guanidine HCl
- Urea or sodium chloride greater than 1M
- Reducing agents DTT, DTE, or 2-mercaptoethanol
- TWEEN 20 or TRITON X-100 greater than 5%
- IGEPAL CA-630 or CHAPS greater than 0.1%
- Digitonin greater than 0.2%
- Deoxycholate or SDS

g. FLAG® Positive Control Lysate is suggested for use as a control in western blotting assays in conjunction with Anti-DYKDDDDK Monoclonal Antibody HRP Conjugate.

VI. Affinity Gel Regeneration

Anti-DYKDDDDK Affinity Gel can be regenerated immediately after use by washing with 3 gel volumes of 0.1 M glycine HCI, pH 3.5. The affinity gel must be immediately re-equilibrated with 10 gel volumes of 0.02M Potassium Phosphate, 0.15M Sodium Chloride pH 7.2 with 0.01% (w/v) sodium azide (added as a preservative). Store the used affinity gel at 4 °C.