

Anti-DYKDDDDK IP Resin

Cat. No. L00425**Technical Manual No. TM0621****Version 01062011**

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1. Product Description

1.1 Designed Use

Anti-DYKDDDDK IP Resin, with high specificity and affinity to DYKDDDDK tag, is ideal for quick and efficient immunoprecipitation of DYKDDDDK-tagged protein or DYKDDDDK-tagged protein-target protein complex.

1.2 Principle

Add the sample containing the DYKDDDDK-tagged protein to the Anti-DYKDDDDK IP Resin and allow the protein bind to the Resin during a short incubation. Then the DYKDDDDK-tagged protein can be eluted off the beads, or used directly for pull-down of target protein.

1.3 Description of Material

Material Supplied

GenScript Anti-DYKDDDDK IP Resin is an agarose beads coupled to Mouse anti-DYKDDDDK monoclonal antibody (clone 6H8E4). The antibody recognizes N-terminal, internal and C-terminal fusion protein containing DYKDDDDK tag. It is supplied as a suspension in 50 % glycerol containing 10 mM sodium phosphate, 150 mM sodium chloride, pH 7.4, 0.02 % (w/v) sodium azide (PBS/A).

Storage and Stability

Store the resin: Anti-DYKDDDDK IP Resin is supplied at -20°C. Do not store the gel at freezing temperatures in the absence of glycerol. Storing the Column: Wash the column three times with 5 ml of TBS/A (TBS containing 0.02 % sodium azide) then add another 5 ml of TBS/A and store at 2-8°C without draining.

Additional Buffers Needed

Equilibration buffer: 50mM Tris, 150mM NaCl, pH 7.4

1XSDS Sample Buffer: 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue

Table 1. Characteristics of Anti-DYKDDDDK IP Resin

Resin Volume	1 ml settled resin (2 ml 50% slurry)
Ligand	Anti-DYKDDDDK mAb generated mouse ascites
Number of DYKDDDDK-tagged protein per ligand	2
M.W. of ligand	Approximately 150 kDa
Degree of substitution	Approximately 8.5 mg Anti-DYKDDDDK mAb /ml settled resin
Storage solution	1X PBS containing 50 % glycerol
Storage conditions	-20°C
Shelf life	12 months when stored unopened

2. Instruction For Use

2.1 Preparation of Cell Lysate

Many different ways may be used for preparing a cell lysate containing expressed DYKDDDDK-tagged proteins. We recommend the use of Easy BacLysis Protein Extraction Solution (GenScript Cat. No. L00230) to prepare a cell lysate from *E.coli*. The other alternative lysis strategies such as French Press and sonication also can be applied for *E.coli*.

2.2 Resin Application

For immunoprecipitation use

It is recommended to use 40 μ l of resin slurry (containing 20 μ l packed resin) per reaction, but this may be scaled up and down as required.

Immunoprecipitation protocol

- 1) Suspend the resin in the vial and immediately transfer 40 μ l of the resin slurry to a microcentrifuge tube.
- 2) Add 1 ml of Equilibration buffer into the tube and invert the tube several times and centrifuge the resin at 5,000 \times g for 30 seconds and remove the supernatant with a pipette. Repeat this step three times.
- 3) Add 200-1000 μ l of the sample to the resin. Gently invert tube several times to resuspend the Resin with sample.
- 4) Incubate the tube on a shaker for 2 hours at room temperature or overnight at 4°C.
- 5) Centrifuge the resin for 30 seconds at 5,000 \times g and remove the supernatants with a pipette.
- 6) Add 1 ml of Equilibration buffer into the tube and invert the tube several times and centrifuge the resin at 5,000 \times g for 30 seconds and remove the supernatant with a pipette. Repeat this step three more times.
- 7) Add 20 μ l of 1 X SDS-PAGE loading buffer into the tube and then heat the tube at 100°C for five minutes.
- 8) Centrifuge the resin at 5,000 \times g for 30 seconds. Transfer the supernatant to a new tube.
- 9) Analyze the sample by SDS-PAGE and Western blot analysis.

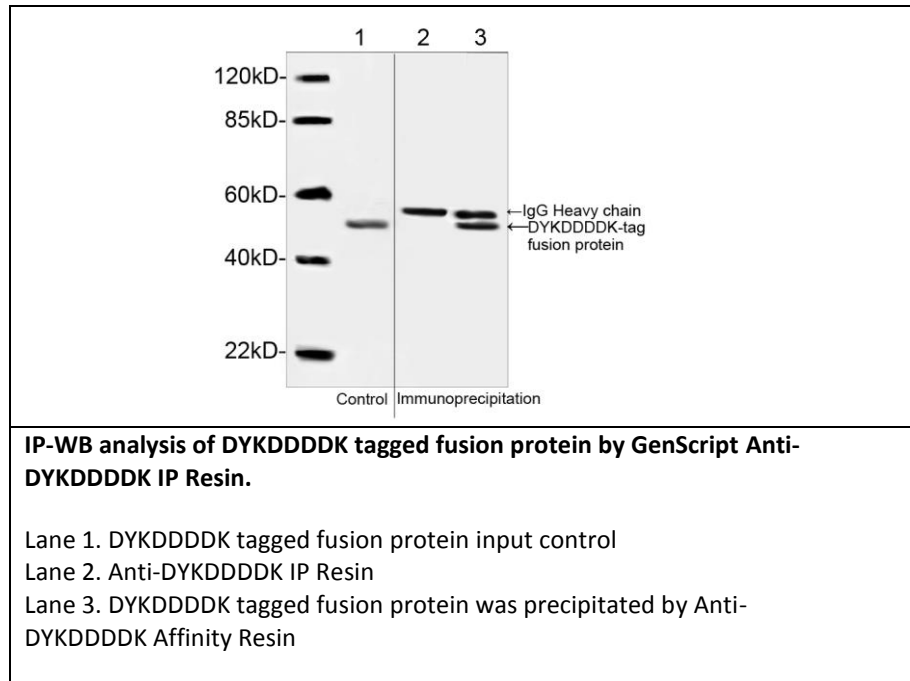
NOTE: Shake or vortex beads vigorously before use.

3. Troubleshooting

Review the information below to troubleshoot your experiments using the GenScript Anti-DYKDDDDK IP Resin.

Problem	Possible Cause	Solution
Multiple non-specific bands observed in the eluted sample	Not enough Equilibration buffer is used.	Increase the volume of Equilibration buffer
	Nonspecific hydrophobic or other interaction.	Increase the concentration of NaCl in the Equilibration buffer
		Add 0.01% Tween-20 or Triton X-100 to the Equilibration buffer.
	The target protein degraded	Add protease inhibitors to Equilibration buffer.

4. Example



5. General Information

5.1 Technical Support

Please contact GenScript for further technical information (see contact details). Certificate of Analysis/Compliance is available upon request. The latest revision of the package insert/instructions for use is available on www.genscript.com.

5.2 Warning and Limitations

This product is for research use only. Not intended for any animal or human therapeutic or diagnostic use unless otherwise stated. Material Safety Data Sheet (MSDS) is available at <http://www.genscript.com>.

5.3 Related Resin Products

Cat. No.	Product Name
A00187	THE™ DYKDDDDK Tag Antibody (Mouse)
A01428	THE™ DYKDDDDK Tag [HRP] Antibody (Mouse)
A01429	THE™ DYKDDDDK Tag [Biotin] Antibody (Mouse)
A01632	THE™ DYKDDDDK Tag [FITC] Antibody (Mouse)
A00170	DYKDDDDK-tag Antibody, pAb, Rabbit
L00405	Chicken IgY Precipitating Resin

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