MonoRab™ Anti-DYKDDDDK Magnetic Beads **Cat. No. L00835**

Technical Manual

I. Product Description

1. Intended Use

GenScript MonoRab™ Anti-DYKDDDDK Magnetic Beads are developed and optimized for protein purification.

2. Principle

The sample containing DYKDDDDK fusion proteins is added to the MonoRab™ Anti-DYKDDDDK Magnetic Beads and incubated on a shaker for a short time for binding. The DYKDDDDK fusion proteins bound to the beads can be eluted by using alkaline elution buffer, competitive elution buffer or PAGE gel sample buffer. Magnetic separation eliminates the need for pre-cleaning of the sample by centrifugation, minimizes the loss of sample, and makes the process more user friendly.

3. Description of Material

3.1 Material Supplied

GenScript MonoRab™ Anti-DYKDDDDK Magnetic Beads are super paramagnetic beads with diameter of 70 µm, covalently coated with a rabbit monoclonal anti-DYKDDDDK antibody. The beads are supplied as 50% slurry in TBS with 50% glycerol and 0.02% sodium azide. The MonoRab™ Anti-DYKDDDDK Magnetic Beads have a binding capacity of 1.5 mg DYKDDDDK-tagged protein (Size: 50KDa) per 1 ml settled beads. The beads can be re-used for at least 5 times.

Based on our new rabbit monoclonal antibody technology (MonoRab™), this beads offers high binding capacity, high purity, and withstand stringent washing steps, especially with buffers containing high concentration of salt. The beads are resistant to up to 2 M NaCl, and to buffers with pH as low as 2.5.

3.2 Additional Material Required

- Mixing/Rotation Device
- Magnetic Separation Rack (L00722 for AmMag™ MR-mini and L00723 for AmMag™ MR)
- Test tubes and pipettes
- Micropipettors
- Microcentrifuge tubes
- Reagent reservoirs
- Serological pipettes (5 ml, 10 ml)
- Protease inhibitor reagents
- Buffers (Table 1)

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Table 1. Necessary Buffers

Buffers	Formulation
Lysis buffer	50 mM Tris-HCl, 150 mM NaCl, pH 7.4, 1mM EDTA, 0.5-1% TritonX-100, 10% Glycerol
Washing buffer	50 mM Tris-HCl, 150 mM NaCl, pH 7.4, 0.5% Tween 20
Loading buffer	1x LDS Buffer (Cat# M00676)
Alkaline elution buffer	0.1 M Tris, 0.5 M NaCl, pH 12.0
Competitive elution buffer	DYKDDDDK peptide in TBS with concentration of 100-500 µg/ml

II. Instruction for Use

1. Sample Preparation

For optimal results, follow the recommendations below for sample preparation.

1) Prepare the sample according to the protein's biophysical characteristics. Optimize lysis conditions to minimize factors interfering with protein binding (See *Reagent Compatibility Table*).
2) To prevent protein degradation during the purification process, perform sample preparation on ice and/or add protease inhibitors to the sample during cell lysis.
3) During cell lysis, add endonucleases to reduce sample viscosity caused by the release of chromosomal DNA or RNA.
4) Avoid frequent freeze-thaw cycles. Make lysate/sample aliquots and store at -80°C.

2. Magnetic Beads Preparation

1) This example protocol uses 40 µl of MonoRab™ Anti-DYKDDDDK Magnetic Beads for routine immunoprecipitation, but this may be scaled up or down as required.
2) Suspend the beads in the bottle thoroughly and transfer the required amount of magnetic beads into an empty tube.
3) Place the tube into a magnetic separation rack for 5-10 seconds to collect the beads and discard the supernatant from the tube with pipette.
4) Add 5 volume of equilibration buffer to the tube and invert the tube several times to wash the beads.
5) Place the tube into the magnetic separation rack for 5-10 seconds and remove the supernatant from the tube with pipette.
6) Repeat the steps 3 to 5 for 3 times, make sure that beads are equilibrated completely.

3. Purification Protocol

1) Add the sample to the prepared magnetic beads. If the sample volume is less than 1 ml, add lysis buffer or TBS to make the total sample volume to 1 ml.
2) Mix by rotation on a tube rotator for at least 1 hour at room temperature.
3) Note: For optimal binding, the incubation time can be extended. If the protein is highly unstable, lowering the incubating temperature is recommended (for example place rotator into the cold room).
4) Place the tube into the magnetic separation rack for 5-10 seconds to collect the beads against the side of the tube. Remove and discard the supernatant with pipette.
5) Add 1ml lysis buffer or TBS to the beads.

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6) Place into the rack to bind the beads and remove the supernatant.

7) Repeat steps 5 and 6 two more times.

8) Elute with alkaline elution buffer, pH 12.0

Add 60 µl (3 magnetic bead volumes) of alkaline elution buffer into the washed beads and use a wide bore pipette tip to gently re-suspend the beads. Incubate at room temperature for 5 minutes, mix gently by tapping the tube once or twice during the incubation period. After incubation, place the tube into the magnetic separation rack for 5-10 seconds to collect the beads against the side of the tube. Carefully transfer supernatant into a new vial containing 3 µl 1M HCl for neutralization.

9) Elution with competitive elution buffer

Add 60 µl (3 magnetic bead volumes) of 100-500 µg/ml DYKDDDDK peptide elution buffer into the washed beads and use a wide bore pipette tip to gently re-suspend the beads. Incubate at room temperature for 5 minutes, mix gently by tapping the tube once or twice during the incubation period. After incubation, place the tube into the magnetic separation rack for 5-10 seconds to collect the beads against the side of the tube. Carefully transfer supernatant into a new vial for further application.

10) Elution with PAGE gel sample buffer

In order to minimize the denaturation and elution of the MonoRab™ antibody immobilized on the beads, no reducing reagents (β-mercaptoethanol or DTT) should be included in the sample buffer. Reducing reagents will dissociate the heavy and light chains of the antibody. If reducing condition are absolutely necessary, a reducing agent may be added. Please refer to *Section IV Reagents Compatibility, Table 4.* Add 50 µl of 1x LDS loading buffer to the tube and heat it at 100 °C for 5 minutes. Centrifuge at 8,000 × g for 30 seconds. Carefully transfer supernatant into a new vial for future application. Please note that if the elution is performed with SDS sample buffer the beads cannot be re-used.

11) Perform analysis immediately or store the sample at appropriate temperature for future analysis.

4. Regeneration of MonoRab™ Anti-DYKDDDDK Magnetic Beads

The MonoRab™ Anti-DYKDDDDK Affinity Beads can be reused multiple times if eluted with alkaline elution buffer or competitive elution buffer, the beads must be regenerated using the following protocol:

6.1 Wash the beads with of 0.1 M Tris HCl, 0.5 M NaCl, pH 12.0.

6.2 Re-equilibrate the beads with of TBS.

6.3 For short-term storage, the beads can be stored in TBS containing 0.02% sodium azide at 2-8 °C, or stored in TBS containing 50% glycerol and 0.02 sodium azide at -20 °C. For most cases, the beads can be stored for a month in these conditions.

The beads can typically be re-used for at least 5 times with minimum loss of binding capability (except if the elution is performed with SDS sample buffer).

III. Reagents Compatibility Table

The tolerable concentration of listed reagents are tested by addition of listed reagents at indicated concentrations.

Table 2. Reagents Compatibility

Reagent	Maximum Tolerable concentration	Note
EDTA	50 mM	Higher concentration of chelating agent will reduce purification efficiency with less target protein recovery.

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β-ME	5 mM	Reducing agents will reduce disulfide bonds in the antibody on the beads. Avoid reducing agents or keep at low concentration (<5 mM) during purification process. When the reducing agents reach the maximum tolerable concentration mentioned here, there will be a band of antibody heavy chain (～50 kDa) in SDS-PAGE analysis. The beads cannot be reused if samples containing higher concentration of reducing reagents are applied to the beads.
DTT	5 mM	
Tween 20	10%	The concentration of detergents should not exceed 10%.
Triton X-100	10%	
SDS	Not suggested	This detergent will denature the MonoRab™ Anti-DYKDDDDK antibody on the beads.
NP-40	10%	Higher concentration will reduce purification efficiency with less target protein recovery.
HCl	pH 2.5	More acid will reduce the binding capacity of the beads by destroying the MonoRab™ Anti-DYKDDDDK antibody on the beads.
Glycerol	20%	Higher concentration will interfere with the binding of DYKDDDDK-tagged protein.
NaCl	2 M	Higher concentration will reduce purification efficiency with less target protein recovery.

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IV. Troubleshooting

Problem	Possible Cause	Solution
Large amount of DYKDDDDK-tagged protein found in supernatant	Binding time is not enough	Increase the incubating time experimentally;
	The DYKDDDDK-tagged protein is overloaded	Reduce the amount of the sample added to the beads or increase the amount of beads.
	DYKDDDDK-tag is not accessible to beads.	Expose the epitope tag by adding low amount of denaturant to the protein extract (dialysis may be needed before applying onto beads), or fuse DYKDDDDK tag to the other terminus of the target protein.
	Beads has not been regenerated since last purification.	Perform beads regeneration procedure prior to binding.
	Reagent compatibility problem	Refer to the reagent compatibility table, and dialyze the sample against TBS before experiment.
Very few or no DYKDDDDK-tagged protein exists in the eluate.	The target protein has been degraded.	1. Use freshly prepared sample 2. Perform purification at lower temperature, such as 4 °C 3. Include protease inhibitors to the sample during cell lysis and binding steps.
	Protein is not completely eluted	Change or optimize the elution methods according to the instructions.
	No target protein expressed	Confirm the presence of target DYKDDDDK-tagged protein in cell lysate with Western blot before purification.
	Very low protein expression level	1. Use larger volume of cell lysate. 2. Optimize expression conditions to raise the protein expression level.
Multiple protein bands found in the eluate.	The protein is not stable at room temperature.	Purify the target protein at lower temperature, such as 4 °C.
	Protein degradation due to proteases activity during purification process	Add protease inhibitors to the cell lysate.
	Non-specific binding	1. Prepare cell lysate again. 2. Add additional wash steps.

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V. General Information

1. Storage and Stability

This product is stable when store at -20 °C for up to 12 months. Drying will cause loss of binding capacity and result in reduced performance. Resuspend the beads well before use.

2. 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 http://www.genscript.com..

3. Warning and Limitations

This product is for research use only. Not intended for any animal or human therapeutic or diagnostic use unless otherwise stated. This product contains sodium azide as a preservative. Classification: Sodium azide <0.1%. Not hazardous at this concentration. The classification was made according to Globally Harmonized System of Classification and Labeling of Chemicals (GHS). Material Safety Data Sheet (MSDS) is available at http://www.genscript.com.

4. Related Products

- A00187 THE™ DYKDDDDK Tag Antibody, mAb, Mouse
- A01428 THE™ DYKDDDDK Tag Antibody [HRP], mAb, Mouse
- A01429 THE™ DYKDDDDK Tag Antibody [Biotin], mAb, Mouse
- A01632 THE™ DYKDDDDK Tag Antibody [FITC], mAb, Mouse
- A01868 MonoRab™ DYKDDDDK Tag Antibody, mAb, Rabbit
- A01869 MonoRab™ DYKDDDDK Tag Antibody [HRP], mAb, Rabbit
- A01870 MonoRab™ DYKDDDDK Tag Antibody [Biotin], mAb, Rabbit
- A01871 MonoRab™ DYKDDDDK Tag Antibody [FITC], mAb, Rabbit
- A00170 DYKDDDDK-tag Antibody, pAb, Rabbit
- L00432 Anti - DYKDDDDK G1 Affinity Beads
- L00766 MonoRab™ Anti-DYKDDDDK Affinity Resin
- RP10586 DYKDDDDK Peptide
- L00722 AmMag™ MR-mini Magnetic Racks
- L00723 AmMag ™ MR Magnetic Racks
- L00743 AmMag™ Box

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