Version 03
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Anti-HA IP Resin Cat. No. L00777

Technical Manual

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1. Product Description

1.1 Designed Use

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

1.2 Principle

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

1.3 Description of Materials

Material Supplied

Genscript Anti-HA IP Resin is an agarose beads coupled to THE™ HA Tag Antibody, mAb, Mouse (Genscript, A01244). The antibody recognizes C-terminal, N-terminal, and internal HA tagged-fusion proteins. 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-HA IP Resin is supplied at -20°C.Do not store the gel at freezing temperatures in the absence of glycerol.

Store 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: 50 mM Tris, 150 mM 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

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Table 1. Characteristics of Anti-HA IP Resin

Resin Volume	1 ml settled resin (2 ml 50% slurry)
Ligand	Anti-HA monoclonal antibody against HA tag
Number of HA-tagged protein per ligand	2
Binding capacity	Approximately 1 mg HA-tagged protein (~20kDa) per ml settled resin
Storage solution	1X PBS containing 50 % glycerol
Storage and stability	Store at -20 °C for up to 12 months.
Resin reuse	The resin can be recycled for at least 3 times. If maintained properly, the resin can be reused 10 times with minimum loss of binding capability.

2. Instruction for Use

2.1 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.
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) Sample should not contain any insoluble particles. Filter the sample or centrifuge with high speed for 10-15 min at 4 °C to remove the insoluble materials before binding procedure.
5) Avoid frequent freezing-thawing cycles. Make lysate/sample aliquots and store at -80°C.

2.2 Resin Application

For Immunoprecipitation Use

It is recommended to use 40 µl of resin slurry (containing 20 µl 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 µl 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×g for 30 seconds and remove the supernatant with a pipette. Repeat this step three times.
3) Add 200-1000 µl 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 ×g and remove the supernatants with a pipette.

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6) Add 1 ml of Equilibration buffer into the tube and invert the tube several times and centrifuge the resin at 5,000×g for 30 seconds and remove the supernatant with a pipette. Repeat this step three more times.

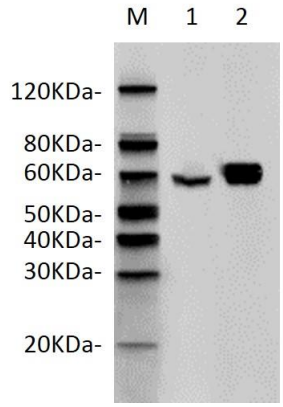
7) Add 20 µl of 1 X SDS-PAGE loading buffer into the tube and then heat the tube at 100°C for five minutes.

8) Centrifuge the resin at 5,000×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. Example

Description	Figure	Figure legend
IP-WB analysis of HA tagged fusion protein by GenScript Anti-HA IP Resin		Lanes: M. Protein Marker 1. Whole cell lysate 2. HA tagged fusion protein was precipitated by Anti-HA IP Resin

4. Troubleshooting

Problem	Possible Cause	Solution
Multiple protein bands found in the eluate.	Non-specific binding	1. Prepare cell lysate again. 2. Add additional wash steps.
	Nonspecific hydrophobic or other interaction	1. Increase the concentration of NaCl in the Equilibration buffer. 2. Add 0.01% Tween-20 or Triton X-100 to the Equilibration buffer.
	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.

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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 www.genscript.com.

5.3 Related Resin Products

Cat.No.	Product Name
A01244	THE™ HA Tag Antibody, mAb, Mouse
A01296	THE™ HA Tag Antibody [HRP], mAb, Mouse
A01297	THE™ HA Tag Antibody [Biotin], mAb, Mouse
A01621	THE™ HA Tag Antibody [FITC], mAb, Mouse
A01806	THE™ HA Tag Antibody [iFluor 488], mAb, Mouse
A01808	THE™ HA Tag Antibody [iFluor 647], mAb, Mouse
A01963	MonoRab™ HA tag Antibody(109B2), mAb, Rabbit
A02002	MonoRab™ HA tag Antibody(109B2)[Biotin], mAb, Rabbit
A00168	HA-tag Antibody, pAb, Goat
A00169	HA-tag Antibody [HRP], pAb, Goat
A00169	HA-tag Antibody [HRP], pAb, Goat
RP11735	HA Peptide

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