

Chicken IgY Precipitating Resin

Cat. No. L00405

Technical Manual No. TM0259

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1. Description

GenScript Chicken IgY Precipitating Resin (GenScript, L00405, 2 ml of 50% slurry) is an agarose beads coupled to affinity purified Goat Anti-Chicken IgY. It displays both high specificity and high affinity for chicken IgY. It is an ideal tool for projects requiring the quick purification of chicken IgY or the enrichment of chicken primary IgY-protein complexes, such as immunoprecipitation (IP) experiments.

GenScript Chicken IgY Precipitating Resin is supplied as a 50% suspension of agarose in 50 mM HEPES, pH 7.6 containing 5 mM EDTA and 0.02% sodium azide.

Table 1. Characteristics of Protein A Resin

Resin Volume	1 ml settled resin (2 ml 50% slurry)
Ligand	Goat Anti-Chicken IgY
Number of IgY binding sites per ligand	2
M.W. of ligand	Approximately 150 kDa
Degree of substitution	Approximately 15 mg IgY /ml settled resin
Static binding capacity	1 – 6 mg chicken IgY/ml bed volume
Matrix spherical	Agarose, 4% cross-linked
Storage solution	50 mM HEPES, pH 7.6 containing 5 mM EDTA
Storage conditions	2-8 °C
Shelf life	12 months when stored unopened

2. General Protocol

A. Additional buffers required

- 1) Washing buffer (pH 7.4): 8.5 g NaCl, 1.4 g Na₂HPO₄, 0.2 g NaH₂PO₄ in 1000 ml Distilled water.
- 2) 1X SDS-PAGE loading 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

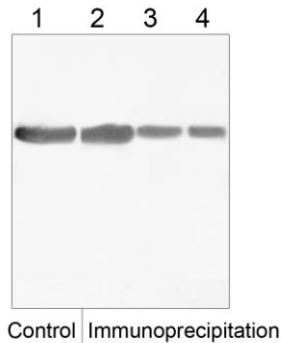
B. Immunoprecipitation procedures

NOTE: Shake or vortex beads vigorously before use.

- 1) Suspend the resin in the vial and immediately transfer 40µl of the resin slurry to a microcentrifuge tube.

- 2) Add 1 ml of Washing 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 200-1000µl 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 hour 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.
- 6) Add 1 ml of Washing 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

3. Example



IP-WB analysis of chicken antibody by GenScript Chicken IgY Precipitating Resin.

Lane 1. Chicken IgY control

Lane 2. Chicken antiserum (5 µl) was precipitated by Chicken IgY precipitating resin

Lane 3. Chicken antiserum (2 µl) was precipitated by Chicken IgY precipitating resin

Lane 4. Chicken antiserum (1 µl) was precipitated by Chicken IgY precipitating resin

At above, 12.5 µl of Chicken IgY Precipitating Resin are used to immunoprecipitate chicken IgY from 5 µl, 2 µl and 1 µl antiserum, respectively. Chicken IgY binding to resin and eluted from resin are analyzed using Goat Anti-Chicken IgY (H&L) [HRP] Polyclonal Antibody (GenScript, A00165) in Western blot.

4. Troubleshooting

Problem	Possible Cause	Solution
No target protein is recovered.	The protein of interest is at a very low concentration in the sample.	Increase the size of the sample.
	The amount of beads is too small.	Increase the amount of beads suspension.
	The binding time is too short.	Increase the binding time.
There are non-specific proteins.	The wash solution is not stringent enough or the volume used is too small.	Increase the concentration of NaCl in the washing buffer.
		Add 0.01% Tween-20 or Triton X-100 to the wash buffer.

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