High-Affinity Iodoacetyl Resin Cat. No. L00403
High-Affinity Antibody Purification Kit Cat. No. L00404

Technical Manual No. TM0392 **Version 07112010**

Index

1. Product Description

GenScript High-Affinity Iodoacetyl Resin is produced by covalently coupling a derivative of iodoacetic acid to 4% cross-linked agarose beads. It is designed for easy and fast immobilization of sulfhydryl-containing peptides, proteins and other ligands. The resin reacts specifically with free sulfhydryls to form stable thioether linkage. The resin is ideal for conjugating sulfhydryl-containing peptide or protein for subsequent affinity purification. 1 ml settled resin can be coupled with more than 1 mg sulfhydryl-containing peptides of 7 amino-acids. Table 1 lists main characteristics of High-Affinity Iodoacetyl Resin.

The High-Affinity Antibody Purification Kit contains High-Affinity Iodoacetyl Resin and all the necessary buffers needed for antibody purification.

Table 1. Characteristics of High-Affinity Iodoacetyl Resin

Resin Volume	10 ml settled resin (20 ml 50% slurry)
Ligand	The derivative of Iodoacetic acid
Binding Capacity	More than 8 mg Rabbit IgG/ml settled resin
pH Stability	pH 5 -10
Support Matrix	4% cross-linked agarose, spherical beads
Mean Particle Size	90 µm
Storage Condition	2°C to 8°C
Shelf Life	12 months when stored unopened

2. Related Products

GenScript also provides High-Affinity Antibody Purification Kit, as the derivative product of High-Affinity Iodoacetyl Resin to facilitate the purification of target antibody.

High-Affinity Antibody Purification Kit Cat. No. L00404

Table 2. Contents of L00403 and L00404

Components	L00403	L00404
High-Affinity Iodoacetyl Resin	10 ml (20 ml 50% slurry)	5 ml (10 ml 50% slurry)
Empty Column		3
5X Coupling Buffer		30 ml
Block Buffer (Solid)		10 ml (adding 10 ml of 1X Coupling Buffer to the solid)
Storage Buffer		20 ml
5X Binding/Wash Buffer		70 ml
Elution Buffer		70 ml
Neutralizing Buffer		15 ml

Note: The buffers must be mixed well before using.

3. Operation

Buffer Preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended filtering the buffers by passing them through a 0.45 μm filter before use.

Coupling Buffer: 50 mM Tris•HCl, 5 mM EDTA-Na, pH 8.5.

Block Buffer: 50 mM L-Cysteine•HCl in Coupling Buffer.

Storage Buffer: Phosphate buffered saline (PBS, pH 8.0) containing 20% ethanol.

Binding/Wash Buffer: Phosphate buffered saline (PBS, pH 8.0).

Elution Buffer: 100 mM glycine•HCl, pH 2.0-2.5.

Neutralizing Buffer: 1 M Tris•HCl, pH 8.5.

4. Protocol for Using High-Affinity Iodoacetyl Resin

A. Coupling Sulfhydryl-containing Sample to High-Affinity Iodoacetyl Resin

1. Sample Preparation. Dissolve the sulfhydryl-containing peptide or protein sample in Coupling Buffer at the concentration of 1 mg/ml.
2. Equilibration. Place the empty column upright in the stand. Transfer appropriate volume of High-Affinity Iodoacetyl Resin into empty column and allow the resin to settle down and buffer to drain. Add 3 bed volumes of Coupling Buffer to the column and allow it to drain at a flow rate of 1 ml/min, repeat this equilibration step two more times.
3. Coupling. Close the flow-speed control and add the sulfhydryl-containing peptide or protein sample. Approximately 1 ml sample solution can be applied to 1 ml High-Affinity Iodoacetyl Resin. Cap the column and incubate on a shaker at room temperature for 30 minutes.
4. Wash. Place the column upright in the stand. Remove top cap and allow the resin to settle down and buffer to drain. Wash column with 3 bed volumes of Coupling Buffer at the speed of 1 ml/min. Collect the flow through and wash to determine the coupling efficiency by comparing the concentrations of the starting sample before and after coupling. The concentration can be measured by absorbance at 280nm or by concentration of the free sulfhydryl group.
5. Blocking. Dissolve the Block Buffer (solid) with 10 ml Coupling Buffer and mix well. Close the flow-speed control and

add dissolved Block Buffer (1 ml Block Buffer can be applied to 1 ml High-Affinity Iodoacetyl Resin). Cap the column and incubate the column on a shaker at room temperature for 30 minutes. After incubation, place the column upright in the stand, remove the cap and allow the resin to settle and buffer to drain.

6. Storage. For immediate use, go to section B. For future use, wash the column with 3 bed volumes of Binding Buffer. The resin can be stored in 1 bed volume of Storage Buffer at 4℃.

B. Purification of Antibody using High-Affinity Iodoacetyl Resin

1. Equilibration. Add 5 bed volumes of Binding/Wash Buffer to the column and allow the resin to settle down and buffer to drain.
2. Antibody Binding. Transfer the antibody sample onto the column with the flow-speed control closed. After sample loading, open the flow-speed control and allow the column to run at the flow rate of about 0.5 ml/min, save the flow through.
3. Wash. Wash the resin with 50 bed volumes of Binding/Wash Buffer at the flow rate of 1.5 ml/min, or until the absorbance of wash at 280 nm is stable.
4. Elution. Elute the antibody with 5 - 10 bed volumes of Elution Buffer at the flow rate of 1 ml/min. Monitor protein content by measuring absorbance at 280 nm. Immediately neutralize the eluted fractions with Neutralizing Buffer. 0.5ml Neutralizing Buffer is needed to neutralize 5 ml eluate.
5. Regeneration of Resin. Wash the resin with 5 bed volumes of Binding/Wash Buffer to remove all Elution Buffer. Store the resin in 1 bed volume of Binding/Wash Buffer containing 20% ethanol at 4°C. **Do not freeze the resin**.

Storage

Store High-Affinity Iodoacetyl Resin in 1 M sodium chloride at 2-8°C in the dark. **Do not freeze.** All the buffers should be stored at 2-8°C. All the other materials are stored at room temperature.

5. Troubleshooting

Problem	Possible Cause	Solution
Protein/peptide precipitates in Coupling Buffer	Protein/peptide is not soluble in Coupling Buffer.	Dissolve sample in ≤ 30% DMSO or DMF or 6M guanidine•HCl in Coupling Buffer.
Low coupling efficiency	Sulfhydryl groups are not free, they are oxidized.	Reduce protein/peptide with DTT or TCEP and proceed immediately to coupling procedure after desalting to prevent reformation of disulfide bonds.
The purity of elution fraction is low	The column was not washed thoroughly.	Increase the volume of Binding/Wash Buffer
Column flows exceedingly slow	Air bubbles in column	Remove air bubbles from the column by stirring the resin or tapping the column gently.

6. Ordering Information

High-Affinity Iodoacetyl Resin	Cat. No. L00403
High-Affinity Antibody Purification Kit	Cat. No. L00404

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