INSTRUCTION MANUAL

SERVA Streptavidin Agarose Resin

AgaroseResin for Affinity Purification of Biotinylated Biomolecules

(Cat. no. 42178)

SERVA
serving scientists

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1. SERVA Streptavidin Agarose Resin

1.1. General information

Streptavidin agarose resin is optimized for the purification of biotinylated biomolecules. The following protocols are general guidelines only. Conditions should be optimized for each application.

1.2. Storage conditions

Store at +2 °C to +8 °C (35 °F – 46 °F). Do not freeze.
If stored at the recommended temperature, the product will be suitable for use until: see label.

2. Immobilization of biotinylated biomolecules using gravity flow affinity columns

2.1. Elimination of the preservative

- Shake the bottle of streptavidin agarose resin suspension gently to get a homogeneous suspension.
- Immediately pipette the suspension into an appropriately sized column.
- Remove first the upper and the lower cap of the column, to allow elimination of the preservative by gravity flow.

2.2. Equilibration of the agarose resin

Binding buffer:
20 mM Na_2HPO_4 (SERVA cat. no. 30200), 150 mM NaCl (SERVA cat. no. 30183), pH 7.4

- Add 5 to 10 bed volumes of binding buffer to agarose resin.
- Mix gently to get a homogeneous suspension.
- Sediment the resin by centrifugation (5 min at 500x g).
- Decant the supernatant carefully and discard it.
- Repeat the equilibration step 2 times.
- A 50 % (v/v) suspension of the pre-equilibrated resin may be used directly or stored at + 4 °C (39 °F) for up to 1 month.

2.3. Sample application

- Close the column outlet.
- Add the sample to the equilibrated resin.
- Close the column inlet.
- Mix the suspension gently at room temperature for minimum 30 min.
- In some cases, a slight increase of contact time may facilitate binding.
- Remove the lower cap of the column, collect the flow through and discard it.

2.4. Washing of the agarose resin

- Close the column outlet.
- Add 10 bed volumes of binding buffer to agarose resin.
- Close the column inlet.
- Mix gently to get a homogeneous suspension.
- Remove the lower cap of the column, collect the flow through and discard it.
- Repeat the washing step until the absorption value reaches a base line.

2.5. Elution

Elution buffer:
8 M Guanidine-HCl (SERVA cat. no. 24205), pH 1.5

- Close the column outlet
- Add 1 bed volume of elution buffer.
- Close the column inlet.
- Mix thoroughly for 10 min at room temperature.
- After sedimentation of the resin, remove the lower cap of the column, collect the flow through and store on ice.
- Repeat the elution step at least 2 times.
- Determine the protein content of each fraction by absorption measurement.
- Pool the fractions containing the sample and immediately dialyze or desalt the sample if needed for downstream applications.

Alternative procedure: Boil the resin in 2 % SDS with 400 mM urea.
This will also dissociate streptavidin monomers.

3. Batch immunoaffinity purification of proteins

Prior to the affinity purification the protein of interested is precipitated with the biotinylated antibody. Afterwards the antigen-antibody complex is incubated with the streptavidin agarose resin for binding.

3.1. Forming of the immune complex

Note: The amount of antigen and the incubation time strongly depends on the antigen-antibody pair. Therefore, it may be necessary to optimize both.

Binding buffer:
20 mM Na_2HPO_4 (SERVA cat. no. 30200), 150 mM NaCl (SERVA cat. no. 30183), pH 7.4

- Solubilize antigen in 50 µl of binding buffer and add the biotinylated antibody in a 1.5 ml tube.
- Adjust the sample volume to 0.2 ml with binding buffer.
- Incubate the sample for 3 – 4 h to overnight at +4 °C.

3.2. Equilibration of the agarose resin

Binding buffer:
20 mM Na_2HPO_4 (SERVA cat. no. 30200), 150 mM NaCl (SERVA cat. no. 30183), pH 7.4

Important: Use approx. 3 mg biotinylated antibody per ml settled streptavidin agarose resin.

- Shake the bottle of Streptavidin agarose resin suspension gently to get a homogeneous suspension.
- Mix the streptavidin agarose resin to ensure an even suspension.
- Immediately pipette the suspension to an appropriate tube.
- Centrifuge for 1 – 2 min at 1,000x g.
- Remove the supernatant carefully and discard it.

3.3. Binding of the immune complex to the agarose resin

- Pipette the appropriate amount of resin into the tube containing the antigen-biotinylated antibody mixture.
- Incubate the sample with mixing for 1 h at room temperature or at +4 °C.

3.4. Washing of the agarose resin

Binding buffer:
20 mM Na_2HPO_4 (SERVA cat. no. 30200), 150 mM NaCl (SERVA cat. no. 30183), pH 7.4

- Wash the resin-bound complex with 0.5 – 1.0 ml of binding buffer.
- Centrifuge for 1 – 2 min at 1,000x g.
- Remove the supernatant.
- Repeat the washing step at least 4 times and remove the final wash.

3.5. Elution

Elution buffer:
100 mM Glycine (SERVA cat. no. 23390)-HCl, pH 2.5

- Add elution buffer to the resin and mix gently.
- Centrifuge for 1 – 2 min at 1,000x g.
- Immediately transfer the supernatant into another tube containing 1 M Tris, pH 7.5 – 9.0 (100 µl/1 ml supernatant).

Alternative procedure: Boil the resin-bound complex in SDS PAGE sample buffer.

4. Immunoaffinity purification of a protein using gravity flow affinity columns

The first step of this purification method is the binding of the biotinylated antibody on the streptavidin agarose resin. Afterwards, the resin-bound antibody is incubated with the sample containing the protein of interest for binding.

4.1. Equilibration of the agarose resin

Binding buffer:
20 mM Na_2HPO_4 (SERVA Cat. No. 30200), 150 mM NaCl (SERVA Cat. No. 30183), pH 7.4

- Shake the bottle of streptavidin agarose resin suspension gently to get a homogeneous suspension.
- Mix the streptavidin agarose resin to ensure an even suspension.
- Immediately pipette the suspension in an appropriate column.
- Wash with 5 – 10 column volumes of binding buffer.

4.2. Antibody binding

- Close the column outlet.
- Apply the biotinylated antibody/protein (use approx. 3 mg of biotinylated antibody/ml of settled resin).
- Close the column inlet.
- Keeping sample and resin in contact at least for 1h at room temperature.
- In some cases a slight increase of contact time may facilitate binding.
- Remove the lower cap of the column and collect the flow through and discard it.

4.3. Washing of the agarose resin

- Close the column outlet.
- Add 10 bed volumes of binding buffer to agarose resin.
- Close the column inlet.
- Mix gently to get a homogeneous suspension.
- Repeat the washing step until the absorption A_{280nm} is less than 0.01 – 0.02.

4.4. Sample application

- Close the column outlet.
- Apply the sample containing the antigen onto the column.
- Close the column inlet.
- Mix the suspension gently at room temperature for min. 30 minutes.
- In some cases a slight increase of contact time may facilitate binding.
- Remove the lower cap of the column, collect the flow through and discard it.
- Wash the resin as described in 4.3.

4.5. Elution

Elution buffer:
100 mM Glycine (SERVA Cat. No. 23390)-HCl, pH 2.5)

- Close the column outlet
- Add 1 bed volume of elution buffer.
- Close the column inlet.
- Mix thoroughly for 10 min at room temperature.
- After sedimentation of the resin, remove the lower cap of the column, collect the flow through and store on ice.
- Repeat the elution step at least 2 times.
- Determine the protein content of each fraction by absorption measurement.
- Immediately neutralize the fractions with 1M Tris, pH 8.0 (100 µl/1 ml eluate) and pool them.

Alternative procedure: Elution with 0.1 M acetic acid.

5. Ordering information

Reagents		
Product	**Cat. no.**	**Size**
Na_2HPO_4	30200.01	500 g
KH_2PO_4	26870.01	500 g
KCl	26868.02	1 kg
NaCl	30183.01	1 kg
Guanidine-HCl	24205.02	1 kg
Urea	24524.02 24524.03	1 kg 5 kg
Glycine	23390.02 23390.04 23390.03	500 g 1 kg 5 kg