For life science research only.
Not for use in diagnostic procedures.

Streptavidin Mutein Matrix

Version: 04
Content Version: November 2021

Immobilized mutant streptavidin for purification of biotinylated proteins.

Cat. No. 03 708 152 001	5 ml matrix settled resin volume 10 ml 50% suspension

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	Streptavidin Mutein Matrix	Ready-to-use 10 ml preswollen gel suspension (50%). For 5 ml column bed volume.	1 vial, 5 ml matrix

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	Streptavidin Mutein Matrix	Store at +2 to +8°C. ⚠ ***Do not freeze.***

1.3. Additional Equipment and Reagent required

For preparation of working solutions

- 2 M HCl
- Dipotassium phosphate: $K_2HPO_4 \times 3\ H_2O$
- Potassium phosphate: KH_2PO_4
- Ammonium sulfate: $(NH4)_2SO_4$
- Sodium chloride: NaCl
- Glycine: NH_2CH_2COOH
- D-biotin
- Sodium azide: NaN_3
- Double-distilled water

For batch purification

- Reaction vials
- pH meter
- Vortex
- Laboratory centrifuge
- Pipettes
- SDS-PAGE equipment, blotting equipment

For column purification

- Disposable plastic columns, such as Mobicol from Mobitec with 35 µm lower filter, or equivalent columns from Perbio Science
- Pipettes
- pH meter
- Measuring cylinder (250 ml, 25 ml)
- SDS-PAGE equipment, blotting equipment

For sample preparation and application

- Biotin Protein Labeling Kit*
- PBS*

For processing of purified biotinylated proteins

- Dialysis equipment (optional)
- De-salting column, such as disposable PD-10 De-salting Column

1.4. Application

The Streptavidin Mutein Matrix is highly suitable for the purification of biotinylated proteins.
- The product has been tested for use with *in vitro*-expressed biotinylated proteins.
- Chemically biotinylated proteins can also be purified using the standard protocol with slight variations.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Regeneration of Streptavidin Mutein Matrix

When properly treated, the matrix can be regenerated and reused at least ten times without a significant loss of binding capacity.

Streptavidin Mutein

Recombinant streptavidin technology was used to screen for a mutant streptavidin with reduced binding affinity toward biotin. By substituting three amino acids, a mutein was obtained with a biotin dissociation constant of 1.3×10^{-7} M. The Streptavidin Mutein Matrix provides this mutein immobilized onto crosslinked agarose beads in a highly stable and regenerable form, which allows purification of biotinylated proteins that results in excellent purity and recovery.

Safety Information

The product does not contain hazardous substances in reportable quantities. Nevertheless, always follow standard safety precautions when handling chemicals. Dispose of used reagents in accordance with local regulations.

Working Solution

⚠ ***Prepare the following buffers and solutions before beginning the purification procedures.***

Solution No.	Solution	Composition	Preparation
1	Potassium phosphate buffer	1 M KH_2PO_4	▪ Dissolve 2.04 g KH_2PO_4 in double-distilled water. ▪ Add double-distilled water to a final volume of 15 ml.
2	Potassium phosphate buffer	1 M KH_2PO_4	▪ Dissolve 22.8 g $K_2HPO_4 \times 3\ H_2O$ in double-distilled water. ▪ Add double-distilled water to a final volume of 100 ml.
3	Washing buffer	100 mM potassium phosphate, 150 mM NaCl, pH 7.2	▪ Dissolve 2.19 g NaCl in 100 ml double-distilled water. ▪ Add 5.75 ml of Solution 1 and 19.25 ml of Solution 2. ▪ Add double-distilled water to a final volume of 250 ml.
4	Equilibration buffer	100 mM potassium phosphate, 150 mM NaCl, 400 mM ammonium sulfate, pH 7.2	▪ Dissolve 2.19 g NaCl and 13.2 g ammonium sulfate in 100 ml double-distilled water. ▪ Add 2.13 ml of Solution 1 and 22.88 ml of Solution 2. ▪ Add double-distilled water to a final volume of 250 ml.
5	Equilibration buffer, 3x conc.	300 mM potassium phosphate, 450 mM NaCl, 1.2 M ammonium sulfate, pH 7.2	▪ Dissolve 0.66 g NaCl and 3.96 g ammonium sulfate in 15 ml double-distilled water. ▪ Add 0.40 ml of Solution 1 and 7.10 ml of Solution 2. ▪ Add double-distilled water to a final volume of 25 ml.
6	Elution buffer	100 mM potassium phosphate, 150 mM NaCl, 2 mM D-biotin, pH 7.2	▪ Dissolve 0.44 g NaCl and 24.4 mg D-biotin in 20 ml double-distilled water. ▪ Add 1.16 ml of Solution 1 and 3.86 ml of Solution 2. ▪ Add double-distilled water to a final volume of 50 ml.
7	Regeneration buffer	100 mM glycine, pH 2.8	▪ Dissolve 0.38 g glycine in 40 ml double-distilled water. ▪ Adjust the pH to 2.8 by adding 2 M HCl. ▪ Add double-distilled water to a final volume of 50 ml.
8	Storage buffer	10 mM potassium phosphate, 0.095% NaN_3, pH 7.0	▪ Add 0.195 ml of Solution 1 and 0.305 ml of Solution 2 to 40 ml double-distilled water, and dissolve 48 mg NaN_3. ▪ Add double-distilled water to a final volume of 50 ml.

2.2. Protocols

Batch protocol or column protocol

The Streptavidin Mutein Matrix can be used in either a batch or column protocol, depending on the size of the purification.

If you...	Then...
need small-scale purification,	use the batch protocol: ▪ Example: Purification of biotinylated proteins from 5 RPIME, *in vitro* protein expression and biotinylation reactions. ▪ Up to 125 µg of a monobiotinylated protein (MW 60 kDa) can be purified using a 50 µl matrix.
need to purify larger amounts of biotinylated proteins,	use the column protocol: ▪ Up to 2.5 mg of biotinylated protein (MW 60 kDa) per ml of matrix. ▪ A 1 ml bed volume is typically sufficient for the purification of biotinylated proteins from 5 RPIME, *in vitro* protein expression and biotinylation reactions.

Batch purification

Preparation of Streptavidin Mutein Matrix column

Prepare 50 µl equilibrated, packed gel matrix for each purification.
See section, **Working Solution** *for information on preparing solutions.*

1. Allow the gel suspension to come to +15 to +25°C.

   Gently shake the vial to homogeneously disperse the gel matrix.

2. Pipette 100 µl of gel slurry, corresponding to 50 µl packed gel, into a vial.
   – Use a 1,000 µl pipette tip with a scissors-cut tip (larger tip opening) to ensure the homogeneous transfer of the gel slurry.

3. Briefly spin down the gel for 15 seconds at approximately 2,000 × *g* in a centrifuge; discard the supernatant.

4. Remove the Storage buffer by adding 500 µl Washing buffer (Solution 3) to the gel.
   – Resuspend, then spin down the gel.
   – Discard the supernatant.

5. Repeat the washing step.

6. Equilibrate the gel with Equilibration buffer (Solution 4) by adding 200 µl of Solution 4.
   – Resuspend, then briefly spin down.
   – Discard the supernatant.

Sample application

See section, **Working Solution** *for information on preparing solutions.*
For experimental details of the expression/biotinylation reaction, refer to the instructions for the 5 RPIME, *E. coli* Biotinylation kits. Monobiotinylated proteins produced by the 5 RPIME devices should be purified as follows.

1. Spin down the reaction mixture after completion of the expression/biotinylation reaction; 5 minutes at 15,000 × *g* in a centrifuge.

2. Remove the supernatant containing soluble biotinylated protein.

3. Mix 2 parts supernatant, containing a maximum of 125 µg monobiotinylated protein, with 1 part Equilibration buffer, 3x conc. (Solution 5).

   Using larger amounts of monobiotinylated protein could potentially overload the gel.

4. Add the sample to the prepared Streptavidin Mutein Matrix, gently mix the sample and gel, then incubate for 10 minutes at +15 to +25°C with occasional mixing.

5. Spin down the gel after the incubation period; 15 seconds at 2,000 × *g* in a centrifuge.

6. Remove supernatant (flow through).

   ⚠ ***Avoid the aspiration of gel particles.***

7. Wash the gel with a total of 10 gel bed volumes; 500 µl Washing buffer (Solution 3) in three steps:
   – Separately collect the first and second wash fractions (1 gel bed volume each).
   – Pool wash fractions 3 to 10 (wash fraction 3, 8 gel bed volumes).

   ⚠ ***Avoid the aspiration of gel particles. Collection of these fractions is optional; perform this collection if you wish to determine the overall purification yield in detail. Usually after the wash step with 2 gel bed volumes, most of the contaminating nonbiotinylated protein is removed.***

Elution of biotinylated protein

See section, **Working Solution** *for information on preparing solutions.*
Elute the biotinylated protein from the Streptavidin Mutein Matrix as follows.

1. Apply the first gel bed volume of Elution buffer (Solution 6) to the gel (50 µl).
   – Briefly mix and spin down the gel.
   – Collect the supernatant (1. elution fraction).

2. Apply the second gel bed volume of Elution buffer (Solution 6).
   – Briefly mix and incubate for 10 minutes at +15 to +25°C.
   – Spin down and collect the supernatant (2. elution fraction).

3. Repeat the elution step twice and collect the supernatants.

   Usually an additional incubation step is not necessary and the biotinylated protein is quantitatively recovered in 3 to 4 elution steps.

Column purification

Preparation of Streptavidin Mutein Matrix column

See section, **Working Solution** *for information on preparing solutions.*
Prepare a column with a bed volume of 1 ml per 2.5 mg of biotinylated protein.

1. Allow the gel suspension to come to +15 to +25°C.

   Gently shake the vial to homogeneously disperse the gel matrix.

2. Close the outlet of a suitable disposable plastic column.

3. Pipette 2 ml of gel suspension, corresponding to 1 ml packed gel into the column.

   Use a pipette tip with an opening large enough to ensure the homogeneous transfer of the gel slurry.

4. Allow the gel to settle, then open the outlet to remove the excess Storage buffer.

   Avoid the introduction of air bubbles into the gel bed and ensure that the column does not run dry.

5. Remove the Storage buffer by washing the gel bed with 10 gel bed volumes of Washing buffer (Solution 3, 10 ml).
   – Discard the Washing buffer.

6. Equilibrate the gel with 4 volumes of Equilibration buffer (Solution 4, 4 ml).

7. Close the column outlet.

Sample application

See section, **Working Solution** *for information on preparing solutions.*
For experimental details of the expression/biotinylation reaction, refer to the instructions for the 5 RPIME, *E. coli* Biotinylation Kit. Monobiotinylated proteins produced by the 5 RPIME devices should be prepared as follows.

1. Spin down the reaction mixture after completion of the expression/biotinylation reaction; 5 minutes at 15,000 × *g* in a centrifuge.

2. Remove the supernatant containing soluble biotinylated protein.

3. Mix 2 parts supernatant, containing a maximum of 2.5 mg monobiotinylated protein, with 1 part Equilibration buffer, 3x conc. (Solution 5).

4. Apply this sample onto the prepared Streptavidin Mutein Matrix column.
   – Open the column outlet and allow the sample to slowly and completely penetrate into the gel.
   – Collect the flow through, then close the column outlet.

5. Incubate for 10 minutes at +15 to +25°C.

6. Wash the gel with a total of 10 gel bed volumes; 10 ml Washing buffer (Solution 3) in three steps:
   – Separately collect the first and second wash fractions; 1 gel bed volume each.
   – Pool wash fractions 3 to10 (wash fraction 3, 8 gel bed volumes).

   Collection of these fractions is optional; perform this collection if you wish to determine the overall purification yield in detail. Usually after the wash step with 2 gel bed volumes, most of the contaminating nonbiotinylated protein is removed.

7. Close the column outlet following completion of the wash steps.

Elution of biotinylated protein

See section, **Working Solution** *for information on preparing solutions.*
Elute the biotinylated protein from the Streptavidin Mutein Matrix as follows.

1. Apply the first gel bed volume of Elution buffer (Solution 6, 1 ml) onto the gel.
   – Open the column outlet, allow the Elution buffer to completely penetrate the matrix.
   – Collect the flow through (elution fraction 1).
   – Close the column outlet.
   – Incubate for 10 minutes at +15 to +25°C.

2. Apply the second gel bed volume of Elution buffer (Solution 6).
   – Open the column outlet.
   – Collect elution fraction 2.

3. Repeat the second elution step twice and collect elution fractions 3 and 4.

   Usually an additional incubation step is not necessary and the biotinylated protein is quantitatively recovered after 3 to 4 elution steps.

Purification of chemically biotinylated protein

This protocol provides a procedure for the purification of up to 2.5 mg of chemically biotinylated protein of average size (MW 60 kDa). Monobiotinylated proteins do not bind efficiently to the Streptavidin Mutein Matrix at low ionic strength. This behavior is basically suited to isolate monobiotinylated proteins from multibiotinylated proteins in an initial purification step at zero M ammonium sulfate (mono- and non-biotinylated proteins in flow through). In a second purification step at an ammonium sulfate concentration >0.8 M, monobiotinylated proteins can be purified from nonbiotinylated ones. The standard protocol described is for use in the purification of reaction mixtures aimed at multibiotinylated proteins.

Preparation of Streptavidin Mutein Matrix column

See section, **Working Solution** *for information on preparing solutions.*
Prepare a column with a bed volume of 1 ml per 2.5 mg of biotinylated protein.

1. Allow the gel suspension to come to +15 to +25°C.

   Gently shake the vial to homogeneously disperse the gel matrix.

2. Close the outlet of a suitable disposable plastic column.

3. Pipette 2 ml of gel suspension, corresponding to 1 ml packed gel, into the column.

   Use a pipette tip with an opening that is large enough to ensure the homogeneous transfer of the gel slurry.

4. Allow the gel to settle, then open the outlet to remove the excess Storage buffer.

   Avoid the introduction of air bubbles into the gel bed and ensure the column does not run dry.

5. Remove the Storage buffer by washing the gel bed with 10 gel bed volumes of Washing buffer (Solution 3, 10 ml).
   – Discard the Washing buffer.

6. Equilibrate the gel with 4 volumes of Equilibration buffer (Solution 4, 4 ml).

7. Close the column outlet.

Sample preparation and application

See section, **Working Solution** *for information on preparing solutions.*

For experimental details of the biotinylation reaction, refer to the Instructions for Use for the Biotin Protein Labeling Kit*. The degree of biotinylation is mainly influenced by the reaction pH, temperature, protein concentration, and the stoichiometry of accessible protein amino groups and the activated biotin compound. These factors must be individually optimized.

1. Dialyze or gel filtrate the reaction mixture following the biotinylation reaction to remove excess biotin compound.

   Refer to the Instructions for Use for the Biotin Protein Labeling Kit. A standard phosphate-buffered saline (PBS) can be used for that purpose.*

2. Mix 2 parts dialyzed sample, containing a maximum of 2.5 mg monobiotinylated protein, with 1 part Equilibration buffer, 3x conc. (Solution 5).

3. Apply this sample onto the prepared Streptavidin Mutein Matrix column.
   – Open the column outlet and allow the sample to slowly and completely penetrate the gel.
   – Collect the flow through.
   – Close the column outlet.

4. Incubate for 10 minutes at +15 to +25°C.

5. Wash the column with a total of 10 gel bed volumes of Equilibration buffer (Solution 4, 10 ml) in three steps:
   – Separately collect the first and second wash fractions (1 gel bed volume each).
   – Pool wash fractions 3 to 10 (wash fraction 3, 8 gel bed volumes).

   Collection of these fractions is optional; perform the collection if you wish to determine the overall purification yield in detail. Typically after the wash step with 2 gel bed volumes, most contaminating nonbiotinylated proteins are removed.

6. Close the column outlet following completion of the wash steps.

Elution of biotinylated protein

See section, **Working Solution** *for information on preparing solutions.*

Elute the biotinylated protein from the Streptavidin Mutein Matrix as follows.

Typically, an additional incubation step is not necessary and the biotinylated protein is quantitatively recovered after 3 to 4 elution steps.

1. Apply the first gel bed volume of Elution buffer (Solution 6, 1 ml) onto the gel.
   – Open the column outlet.
   – Let the Elution buffer completely penetrate into the matrix.
   – Collect the flow through (elution fraction 1).
   – Close the column outlet.
   – Incubate for 10 minutes at +15 to +25°C.

2. Apply the second gel bed volume of Elution buffer (Solution 6).
   – Open the column outlet.
   – Collect elution fraction 2.

3. Repeat the second elution step twice and collect elution fractions 3 and 4.

Regeneration of Streptavidin Mutein Matrix

See section, **Working Solution** *for information on preparing solutions.*

Used Streptavidin Mutein Matrix can be easily regenerated in the column using the following procedure.

Following this treatment, the Streptavidin Mutein Matrix is ready to use in the next purification step. Close and seal the column inlet and outlet.

1. Wash with 5 gel bed volumes of Washing buffer (Solution 3).

Protocol	Volume
Batch purification	250 µl
Column purification	5 ml

2. Wash with 5 gel bed volumes of Elution buffer (Solution 6).

Protocol	Volume
Batch purification	250 µl
Column purification	5 ml

3. Wash with 6 gel bed volumes of Regeneration buffer (Solution 7).

Protocol	Volume
Batch purification	300 µl
Column purification	6 ml

4. Wash with 5 gel bed volumes of Equilibration buffer (Solution 4).

Protocol	Volume
Batch purification	250 µl
Column purification	5 ml

Preparation for storage

See section, **Working Solution** *for information on preparing solutions.*

1. Wash the matrix with 10 gel bed volumes of Storage buffer (Solution 8):

Protocol	Volume
Batch matrix	500 µl
Column matrix	10 ml

2. Store the washed matrix at +2 to +8°C in a sealed vial (batch matrix) or the column with a sealed inlet and outlet.

The matrix is stable in this form for several months.

Processing of purified biotinylated protein

The eluted biotinylated protein contains 2 mM D-biotin at pH 7.2. If this is not an appropriate storage buffer or if free D-biotin will interfere with further analysis or applications, for example, binding on SA-coated surfaces, a preceding dialysis or column de-salting step should be performed.

Dialysis of eluted biotinylated protein

- The elution fractions that contain the biotinylated protein are pooled and filled into a micro dialysis device, such as the Slide-A-Lyzer, Pierce 66330, capacity 0.5 to 3 ml; MWCO 3.500; Slide-A-Lyzer Syringe, 1 ml, Pierce 66494. Refer to the corresponding suppliers' usage instructions.
- Choose dialysis buffer volumes that are large enough to ensure the proper dilution of D-biotin for further applications (>1,000 fold of sample volume).
- For the dialysis of very small sample volumes (5 to 100 µl), perform drop dialysis using a V membrane filter of 0.025 µm (Millipore VSWP 01300).

Analysis of purified product

Tracking of purification by analysis on SDS-PAGE followed by western blot is recommended. Aliquots of the following fractions can be analyzed.

- Starting material
- Flow through
- Wash (initial fractions)
- Eluate

If significant amounts of target protein are found in flow through or wash fractions, see section, **Troubleshooting**.

2.3. Parameters

Affinity/Binding Capacity

Matrix capacity

1 ml Streptavidin Mutein Matrix binds at least 2.5 mg of monobiotinylated Fab fragment per ml packed gel (MW approximately 60 kDa).

3. Results

Batch purification of *in vitro* monobiotinylated proteins

Penicillin G Amidase (PGA) and D-Amino Acid Oxidase (DAO) had been expressed and biotinylated from pIVEX2.7dPGA and pIVEX2.7dDAO, respectively, using the RTS 500 ProteoMaster *E. coli* HY Kit and RTS AviTag Biotinylation Kit. 50 µl of supernatant from the expression reaction was purified on a 50 µl Streptavidin Mutein Matrix, see Figure 1. Fractions representing different steps of the purification procedure were analyzed on SDS-PAGE. PGA is a heterodimeric protein consisting of a 63 kDa β-subunit and a 24 kDa subunit, see arrows in Figure 1. DAO is a homodimeric protein consisting of a 38 kDa subunit.

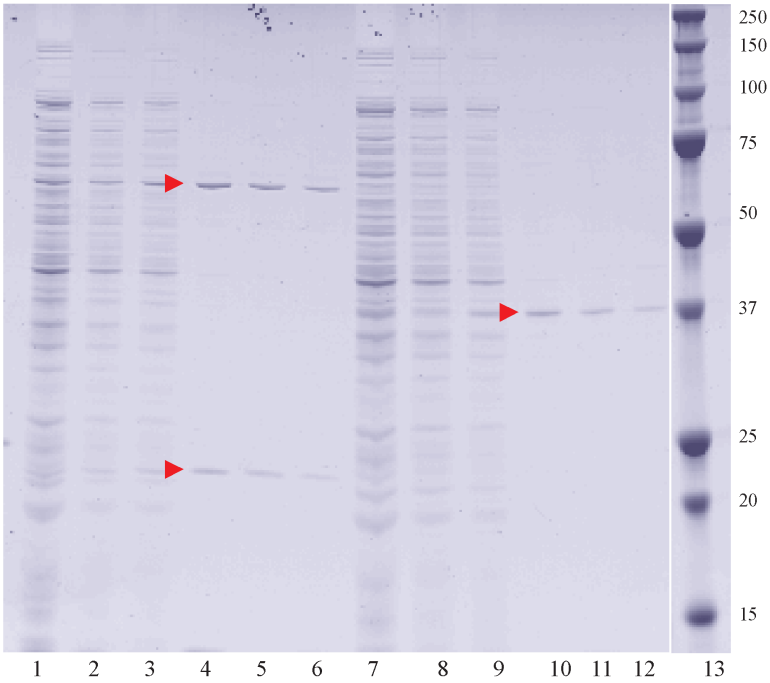

Fig. 1: Purification of biotinylated PGA and DAO on the Streptavidin Mutein Matrix, gel of SDS-PAGE stained with colloidal Coomassie blue. Volume of flow through and wash/elution fractions correspond to one gel bed volume (50 µl).
Lanes 1 to 6: PGA (1: Flow through; 2: Wash 1; 3: Wash 2; 4 to 6: Elutions 1 to 3).
Lanes 7 to 12: DAO (7: Flow through; 8: Wash 1; 9: Wash 2; 10 to 12: Elutions 1 to 3).
Lane 13: MWM
Arrows indicate the subunits of PGA respective to DAO.

Column purification of *in vitro* monobiotinylated chloramphenicol acetyltransferase (CAT)

Chloramphenicol acetyltransferase (CAT) has been expressed and biotinylated from pIVEX2.7dCAT using the RTS 500 ProteoMaster *E. coli* HY Kit and RTS AviTag Biotinylation Kit. The supernatant of the expression reaction was purified on a 2.3 ml Streptavidin Mutein Matrix column. Column was loaded with 2.3 mg biotinylated CAT/ml gel matrix. Fractions representing different steps of the purification procedure were analyzed on SDS-PAGE, see Figure 2. The elution of pure CAT was essentially achieved in two column volumes of Elution buffer.

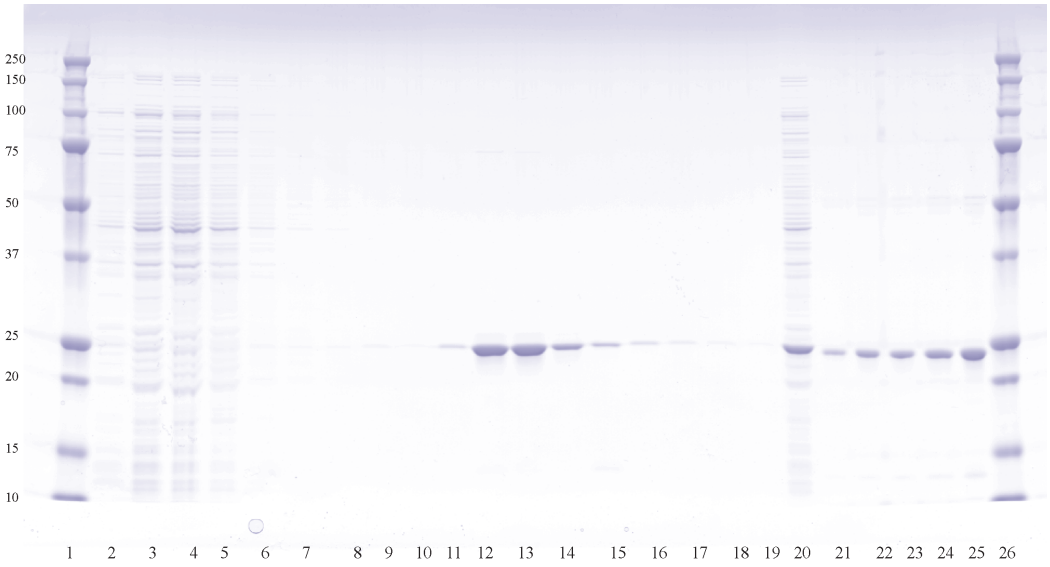

Fig. 2: Purification of biotinylated CAT on Streptavidin Mutein Matrix, gel of SDS-PAGE stained with colloidal Coomassie blue; wash/elution fractions correspond to 0.5 column volume each.
Lane 1: MWM
Lane 2: Flow through
Lane 3 to 8: Wash fractions 1 to 6
Lane 9: Wash fractions 7 to 20 (pool)
Lanes 10 to 19: Elution fractions 1 to 10
Lane 20: Original *in vitro* expression and biotinylation mixture
Lanes 21 to 25: CAT standards 158 ng, 317 ng, 475 ng, 633 ng, and 950 ng
Lane 26: MWM

4. Troubleshooting

Observation	Possible cause	Recommendation
Biotinylated protein appears in flow through.	Column overloaded.	Reduce sample load.
	Binding conditions were not optimal.	In some cases, monobiotinylated proteins may require a higher salt concentration to bind to the mutant streptavidin. Increase ammonium sulfate concentration to 0.6 to 1 M. ⚠ ***Ensure that the protein is not precipitated.***
		Also, wash matrix following sample application with Equilibration buffer that contains 400 mM ammonium sulfate to minimize the loss of monobiotinylated protein during washing.
		Check sample pH before loading onto the column; pH after sample preparation should be approximately 7.2.
		Increase the time allowed for the sample to bind to the matrix.
	Matrix was not efficiently regenerated and/or equilibrated from the previous purification.	Repeat regeneration and equilibration step.
	Column was not properly packed, leading to cracked gel bed (sample channeling).	Repeat column packing; avoid gel bed cracking.
	Concentration of free biotin from biotinylation step was too high, and was thus efficiently competing with biotinylated protein.	Check biotinylation protocol; biotin concentrations in the range of the AviTag biotinylation reaction (150 µM) are not critical for binding.
		Higher concentrations of free biotin should be avoided; reduce free biotin concentrations via dialysis or ultrafiltrate the protein sample.
	Incomplete biotinylation reaction, nonbiotinylated protein in flow through.	Perform a western blot with Streptavidin-Peroxidase staining; ensure proper biotinylation conditions.
	Biotin residue of biotinylated protein was not freely accessible for binding to mutant streptavidin.	Vary binding conditions; reduce or increase total ionic strength.
		Change biotinylation protocol.
		Change AviTag position (C- versus N-terminus).
Biotinylated protein appears in wash fraction.	Stable binding of biotinylated protein may need a continuously higher ammonium sulfate concentration even in the wash step.	Use Equilibration buffer that contains 400 mM ammonium sulfate for the wash step; if needed, increase the ammonium sulfate concentration to 800 or 1,000 mM.

Purified protein in eluate is not homogeneous.	Protein degradation during preparation and/or purification.	Add protease inhibitors during protein preparation; perform purification at +2 to +8°C.
	Internal initiation sites or premature stop may lead to shortened proteins.	Check and correct for internal initiation sites.
	Nonspecific interaction of other proteins in the biotinylation mixture with the matrix.	Check with a western blot and Streptavidin-Peroxidase staining; reduce the concentration of ammonium sulfate in the sample and column preparation step.
Additional Coomassie-stained protein band of MW 14 kDa is visible in SDS/PAGE.	Streptavidin Mutein Matrix particles had been transferred into SDS sample buffer during batch purification. This band indicates that mutant streptavidin subunits were solubilized from the matrix upon boiling in SDS sample buffer.	Avoid the transfer of matrix particles during the elution step of the batch purification.
	Additional biotinylated proteins were present in the sample.	Check for the presence of contaminating proteins, which were also biotinylated in the biotinylation reaction (chemical biotinylation).
		Check for biotinylated lysate components (*in vitro* expression/ biotinylation). For example, *E. coli* lysate could contain minute amounts of BCCP (biotin carboxyl carrier protein, MW 17 kDa).
		Perform a preclearance step with immobilized streptavidin before expression/ biotinylation.
Biotinylated protein cannot be detected in the elution fractions.	Biotinylated protein did not elute, biotin concentration in the Elution buffer was too low.	Check for the proper biotin concentration in the Elution buffer (2 mM).
	Protein was lost in the binding and/or wash step.	Check for protein in flow through and wash fractions.

5. Additional Information on this Product

5.1. Test Principle

How this product works

The purification procedure with the Streptavidin Mutein Matrix is shown in the following steps.

① Matrix equilibration.

② Sample application.

③ Removal of unbound material via washing.

④ Elution of bound biotinylated proteins.

⑤ Matrix regeneration.

⑥ Analysis of purified product with SDS-PAGE, and blotting.

Purification of biotinylated proteins

The binding of biotin to avidin/streptavidin is the strongest known noncovalent interaction of biological molecules (K_d 0.6×10^{-15} M (avidin), 4×10^{-14} M (streptavidin). Tetrameric avidin and streptavidin, although highly dissimilar with regard to overall amino acid composition and sequence, gain their extreme affinity toward biotin via a highly stabilized network of polar and hydrophobic interactions. The overall binding process is accompanied by conformational changes and subunit interactions. Strong binding is advantageous for certain applications of the derived biotin-avidin/streptavidin technology, such as

- Surface immobilization
- Blotting technologies
- Immunoassays
- Crosslinking studies
- Histochemistry

However, the use of immobilized avidin/streptavidin for the purification of biotinylated proteins is limited. In this instance, the extreme affinity of the binding partners creates a drawback of enabling elution only under very harsh conditions that usually destroy the functionality of the protein of interest. A lot of effort has been spent on the development of binder/ligand systems that have reduced affinity (monomeric avidin, monomeric streptavidin, nitrosylated avidin), or a binding behavior, which can be modulated by pH changes (avidin/iminobiotin). In addition, peptide ligands with reversible streptavidin/avidin binding affinity have been screened, but they suffer from serious disadvantages: monomeric avidin tends to reassociate to high affinity, tetrameric avidin; avidin (a fairly basic protein) generally shows a higher degree of nonspecific binding; the use of iminobiotin requires a pH drop, which could denature the protein of interest; and peptide ligands usually depend on specifically designed streptavidin derivatives.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
Biotin Protein Labeling Kit	1 kit, 5 labeling reactions	11 418 165 001
Streptavidin Conjugates	Streptavidin-AP Conjugate, 1,000 U	11 089 161 001
	Streptavidin-POD Conjugate, 500 U	11 089 153 001

6.4. Trademarks

All product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products and select the corresponding product catalog.

6.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed

1121.03730913001 ©

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany