Streptavidin HP MultiTrap

Streptavidin HP MultiTrap™ contains:

- 4 prepacked Streptavidin HP MultiTrap 96-well filter plates
- Instructions for use

Purpose

Small-scale sample preparation for single use: Enrichment of target protein, upstream of gel electrophoresis, liquid chromatography, and mass spectrometry. MultiTrap may be used with robotic systems or manually, with centrifugation or vacuum.

Principles

Streptavidin HP MultiTrap filter plates enables the binding of biotinylated affinity biomolecules (e.g., antibodies, proteins, and aptamers) to Streptavidin Sepharose™ High Performance. The immobilized biomolecule is used to capture target proteins that interact with it. Impurities are removed by washing, and the enriched target protein is eluted. Protein-protein interaction studies can also be performed using this technique.

Recommended buffers

Binding buffer:	TBS (50 mM Tris, 150 mM NaCl, pH 7.5)
Wash buffer:	TBS with 2 M urea, pH 7.5
Elution buffer:	0.1 M glycine with 2 M urea, pH 3.0
Blocking buffer:	2 mM Biotin in TBS

Alternative buffers

Wash buffer:	• 0.1 M triethanoleamine with 0.5 M NaCl, pH 9.0.
Elution buffers:	• 0.1 M glycine, pH 2.5 to 3.5 • 0.1 M citric acid, pH 2.5 to 3.5 • 2% SDS • 0.1 M ammonium hydroxide, pH 10 to 11

For further optimization of the protocol, see "*Optimization Guide*".

Before starting

Centrifugation, incubation and elution:

- Centrifuge the MultiTrap plates at 700 × g or use vacuum. If vacuum is used, apply 0.15 bar until the wells are empty, then slowly increase the vacuum to -0.3 bar (do not apply more vacuum than -0.5 bar). Turn off the vacuum after approximately 5 sec.
- Mix briefly before removal of liquid in the equilibration, wash and elution steps to increase the efficiency of the step. Incubating on a plate shaker is recommended.
- All incubations should normally be performed at room temperature. However, incubations may be performed at lower temperatures when a slower process is preferable (see "*Optimization Guide*" for further information). During incubation, cover the plate using a sealing tape or an appropriate 96-well cover.
- Collection plates are not included and must be ordered separately (see "*Related products*"). Remember to change or empty the collection plate between steps.

Sample pretreatment:

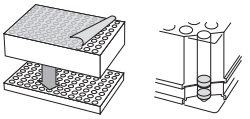
- Excessive cellular debris and lipids may clog the wells. Clarify the sample by centrifugation or filtration before applying to the MultiTrap plate.
- To prevent target protein degradation, inhibition of protease activity may be required (a Protease Inhibitor Mix is available, see "*Related products*").

Protocol

The protocol is designed as a starting protocol for enrichment of target proteins by using immobilized antibodies.

1 Remove storage solution

- Suspend the medium by gently shaking the plate upside down.
- Remove top and bottom seals and place plate on the collection plate.
- Remove the storage solution by centrifugation for 1 min at 700 x g.



Shake gently upside down
Remove the seals
1 min
700 × g

2 Equilibration for immobilization

- Add 400 µl binding buffer per well, mix briefly and centrifuge for 1 min at 700 × g to equilibrate the medium. Perform this step three (3) times total.

3 ×

Add 400 µl
binding buffer
1 min
700 × g

3 Binding of biotinylated antibody

- Immediately after equilibration, add 200 µl of the biotinylated antibody solution per well (0.1 to 1.0 mg/ml in binding buffer).
- Incubate on shaker for 20 min.
- Centrifuge for 1 min at 700 × g to remove unbound antibody.

Add 200 µl biotinylated antibody in binding buffer
Incubate 20 min on shaker
1 min
700 × g

4 Blocking

- Add 400 µl blocking buffer per well and incubate on shaker for 5 min to block free biotin binding sites.
- Centrifuge for 1 min at 700 × g. Perform this step two (2) times total.

2 ×

Add 400 µl blocking buffer
Incubate 5 min on shaker
1 min
700 × g

5 Washing

- Add 400 µl binding buffer per well and mix briefly.
- Centrifuge for 1 min at 700 × g. Perform this step three (3) times total.

3 ×

Add 400 µl binding buffer
1 min
700 × g

6 Binding of target protein

- Add 200 µl sample in binding buffer per well and incubate on shaker for 60 min.
- Centrifuge for 1 min at 700 × g to wash out unbound protein. Collect flowthrough.

Add 200 µl sample in binding buffer
Incubate 60 min on shaker
1 min
700 × g

7 Washing

- Add 400 µl binding/wash buffer per well and mix briefly.
- Centrifuge for 1 min at 700 × g.
  Perform this step five (5) times total.
  (Collect and save washes in case troubleshooting is needed).

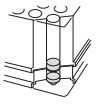
5 ×

Add 400 µl binding/wash buffer
1 min
700 × g

8 Elution

- Add 200 µl of desired elution buffer and shake for 1 min.
- Centrifuge for 1 min at 700 × g.
  Perform this step three (3) times total.
  Collect the eluates in separate collection plates.

3 ×



Add 200 µl elution buffer
Shake for 1 min
1 min
700 × g

Notes

Optimization Guide

- To prevent the wells from clogging the sample may need to be clarified and/or diluted.
- Optimizations may be necessary when using vacuum. If the vacuum causes foam or contamination to rise in the collection plate, increase or decrease the vacuum to improve the situation. Different centrifugation speeds may also be tried.
- Biotinylated antibody/protein may be prepared using different laboratory methods. Ready-made biotinylated antibodies/ proteins are also commercially available.
  - To avoid introducing contaminants, remember to remove non-reacted biotin and by-products by desalting or dialysis prior to use.
- Proteins may also be labelled with 2-iminobiotin, which binds to streptavidin above pH 9.5 and can be eluted at pH 4.
- The time for incubation and amount of antigen are antibody-antigen dependent. Optimization (choice of buffers, number of washes, incubation time) may be required for each specific antigen and antibody to obtain the best results. At room temperature, the reaction is usually completed within 30 to 60 min. If the binding is performed at 4°C, it can be left overnight.
- Protein recovery, specific purity and elution optimizations:
  - Improve the specific purity by adding detergent, different salts, and different concentrations of salts to the wash buffer.
  - Low protein yields may be a result of excessive biotinylation interferes with the antibody-antigen interaction, insufficient biotinylation, or the acidic elution conditions for the studied protein.
  - Lower the concentration of biotinylated antibody to further minimize impurities that may co-elute with the target protein. As an alternative, a preclearing step can be performed before the enrichment procedure. For preclearing, add the sample to a separate MultiTrap containing blocked medium without biotinylated antibody, and incubate for 0.5 to 4 h. Collect the sample and proceed with the standard protocol.
  - For alternative buffers, see table on page 1.
- Streptavidin HP MultiTrap may also be used for simple, small scale enrichment of biotinylated biomolecules.

Characteristics of Streptavidin HP MultiTrap

Matrix	Highly cross-linked agarose 6%
Medium	Streptavidin Sepharose High Performance
Ligand	Streptavidin
Ligand density	10 µmol/ml
Binding capacity[1]	Biotin > 300 nmol/ml gel, Biotinylated Bovine Serum Albumin 6 mg/ml gel
Average particle size	34 µm
pH stability	4 to 9 (long term) 2 to 10.5 (short term)
Working temperature	4 to 30ºC
Storage solution	20% ethanol
Storage temp	4 to 8ºC
Filter plate material	Polypropylene and polyethylene
Filter plate size[2]	127.8 x 85.5 x 30.6 mm
Volume prepacked medium/well	50 µl
Well volume	800 µl
Centrifugation speed[3]	700 x g
Vacuum pressure[3]	
• Recommended	-0.1 to -0.3 bar
• Maximum	- 0.5 bar

[1] Protein dependent

[2] According to American Standard Institute (ANSI) and Society for Biomolecular Screening (SBS) standards 1-2004, 3-2004 and 4-2004.

[3] Actual settings will depend on the sample properties and pre-treatment.

Ordering information

Description	Quantity	Code No.
Streptavidin HP MultiTrap	4 x 96-well filter plates	28-9031-31

Related products

Description	Quantity	Code No.
Sample Grinding Kit	50 samples	80-6483-37
Protease Inhibitor Mix	1 ml	80-6501-23
Nuclease Mix	0.5 ml	80-6501-42
NHS HP SpinTrap	1	28-9031-28
Streptavidin HP SpinTrap™	16 columns	28-9031-30
Protein A HP SpinTrap	16 columns	28-9031-32
Protein A HP MultiTrap	4 x 96-well filter plates	28-9031-33
Protein G HP SpinTrap	16 columns	28-9031-34
Protein G HP MultiTrap	4 x 96-well filter plates	28-9031-35
Collection Plate	5 x 96 well plates	28-4039-43
Ab SpinTrap	50 x 100 µl	28-4083-47
Ab Buffer Kit	1	28-9030-59

Literature

Title	Code No.
Data File Streptavidin HP MultiTrap	28-9067-91
Antibody Purification Handbook	18-1037-46
Affinity Chromatography Handbook	18-1022-29

www.gehealthcare.com/sampleprep

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

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