Purification Protocol for *Strep*-Well HT purification plates

Last date of revision
May 2005

Version: PR04-0003

IBA Headquarters
IBA GmbH
Rudolf-Wissell-Str. 28
D-37079 Göttingen
Germany
Tel: +49 (0) 551-50672-0
Fax: +49 (0) 551-50672-181
info@iba-go.com
www.iba-go.com

IBA US Distribution Center
10748 Indian Head Industrial Blvd.
St. Louis, MO 63132
Tel. 1-877-IBA-GmbH (1-877-422-4624)
Fax 1-888-531-6813
info@iba-go.com
www.iba-go.com

Strep-tag®/*Strep*-Tactin® affinity purification

The *Strep*-tag purification system is based on the highly selective binding of engineered streptavidin, called *Strep*-Tactin, to *Strep*-tag II fusion proteins. This technology allows one-step purification of almost any recombinant protein under physiological conditions, thus preserving its bioactivity. The *Strep*-tag system can be used to purify functional *Strep*-tag II proteins from any expression system including baculovirus, mammalian cells, yeast, and bacteria.
Because of its small size, *Strep*-tag generally does not interfere with the bioactivity of the fusion partner. Thus, removal of the tag becomes superfluous. Comprehensive reviews and scientific publications giving an overview of various *Strep*-tag applications are listed at www.iba-go.com.

Protocol for affinity purification via *Strep*-Well HT purification plates

Perform all operations at a temperature amenable to the stability of your recombinant protein (between 4 °C and 30 °C). At low expression levels, increase applied cell extract volumes to take advantage of the column capacity, without changing other volumes. All buffers included in the kit are provided as 10x solutions and have to be diluted 1:10 with distilled water prior to use.

Vacuum filtration

For one typical vacuum filtration step the following procedure should be performed: Apply vacuum of about 200 mbar for 2 seconds and switch the vacuum at the vacuum manifold off for 8 seconds and repeat this vacuum on/off-cycle 15 times to guarantee the complete liquid passage in all wells of the plate. The number of vacuum cycles may need to be optimized in each individual case for best performance.

Optional:

- Due to different expression levels the viscosity of the extracts – and consequently the time to flow through the plate – might vary for each well. Since flow rates above 2.5 ml/minute should be avoided, it is not recommended to use maximum vacuum. Best results have been obtained when vacuum filtration steps were proceeded as described above followed by a brief application of maximum vacuum to ensure complete liquid removal from each well.
- If the bacterial cultures were grown to high density, the lysates may be too viscous to pass through the plates. Do not allow cultures to reach an OD_{600} higher than 6.
- If not all 96 wells of the plates are used in parallel it is recommended to seal any unused wells with tape.

Equilibration of *Strep*-Well HT purification plates

Equilibrate each well by adding 2 times 1 ml Buffer W (100 mM Tris, pH 8.0, 150 mM NaCl, 1 mM EDTA; use buffer without EDTA for metalloproteins). Perform vacuum filtration for each equilibration step as described above.

Adsorption of the *Strep*-tag II fusion protein

Add between 50 µl and 1 ml cell extract per well (concentrated cell extracts are preferred; if quantification is possible, apply cell extract containing between 5 and 10 nmol recombinant *Strep*-tag II fusion protein per well). Perform vacuum filtration.
Frozen cell extracts have to be centrifuged/filtered before applying them to the plate in order to remove any aggregates that may have formed (microcentrifuge, 14,000 rpm, 5 minutes, 4 °C).

Optional:

- As an alternative to the direct application of centrifuged extract prepared by sonication, the cells could be lysed by enzymatic digestion using lysozyme (1 mg/ml in 100 mM Tris, pH8.0, DNase and protease inhibitors for 30 minutes at 37°C with agitation at 750 rpm). The extract could be cleared by a filtration step using the enclosed filter plate. To clarify the lysate, the *Strep*-Well HT purification plate is placed into the base and the filter plate on top of the vacuum manifold. The spouts of the filter plate should fit into the wells of the *Strep*-Well HT purification plate. To prevent foaming under the spouts of filter plate, which might lead to cross-contaminations, it is recommended to overlay the applied lysates with 0.1 volume ethanol (up to 100 µl) prior to the vacuum filtration step. The *Strep*-tag binding properties will not be affected. Please note that the time allowed to lyse the cells may require optimization.
- If the cell extract is not too viscous protein yield can be increased by an incubation step of 2 minutes allowing the extract to enter the well of the *Strep*-Well HT plate by gravity flow prior to performing vacuum filtration.

Washing of *Strep*-Well HT purification plates
Wash the individual wells 4 times with 0.5 ml Buffer W by vacuum filtration.

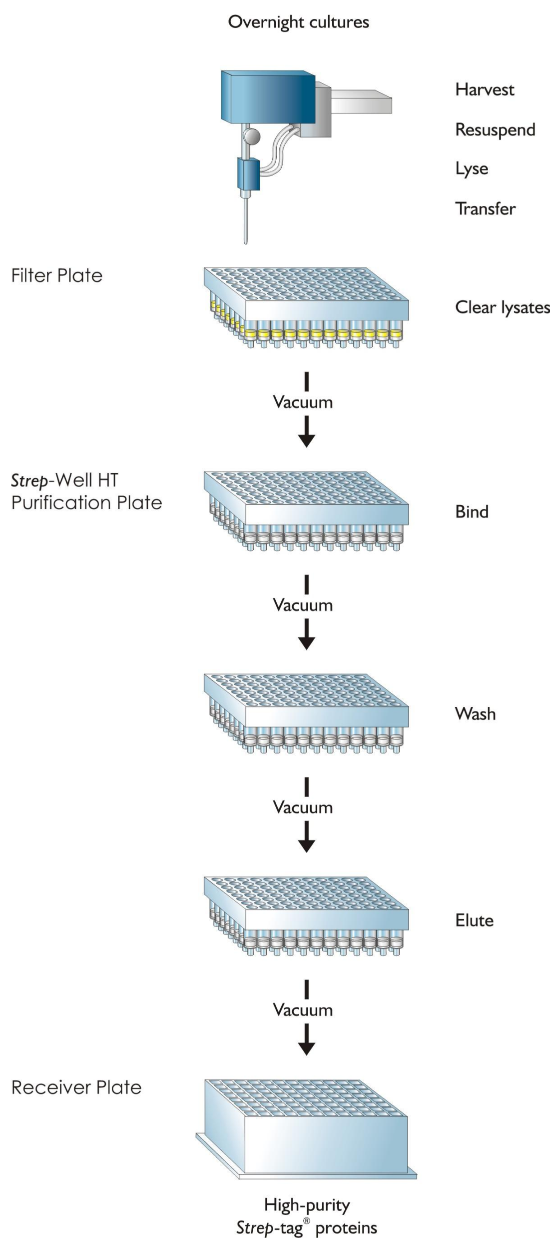
Optional:

- The enclosed wash plate might be used to prevent cross contamination during washing.

Elution of the recombinant protein
Add 3 times 150 µl Buffer E (= Buffer W containing 2.5 mM desthiobiotin as a reversibly binding specific competitor) and collect the eluate in the receiver plate.

Optional:

- If Buffer E is able to enter the well by gravity flow, an incubation step of 2 minutes prior to applying vacuum will increase protein yield. As an alternative to using an elution buffer containing desthiobiotin the recombinant protein can also be eluted by applying 2 mM biotin which will elute more concentrated protein solution but will prevent regeneration of the affinity matrix.



Optional: Regeneration of the *Strep*-Well HT purification plates (only applies when desthiobiotin has been used for elution)

For regeneration, wash the individual wells 3 times with 1 ml Buffer R (= Buffer W containing 1 mM HABA). The color change from yellow to red indicates the regeneration process and the intensity of the red color is an indicator of the resin activity status. Remove Buffer R by adding 2 times 1 ml Buffer W before the next purification run and performing vacuum filtration.

After usage store the 96 well plate at 2-8 °C in humidified environment.

Buffer compositions

Buffer W (washing buffer)	Buffer E (elution buffer)	Buffer R (regeneration buffer)
100 mM Tris-Cl pH 8.0 150 mM NaCl 1 mM EDTA	100 mM Tris-Cl pH 8.0 150 mM NaCl 1 mM EDTA 2.5 mM desthiobiotin	100 mM Tris-Cl pH 8.0 150 mM NaCl 1 mM EDTA 1 mM HABA (hydroxy-azophenyl-benzoic acid)

Related products

Cat. No.	Product
2-1700-000	Strep-well HT 25 Purification Starter Kit
2-1701-000	Strep-well HT 50 Purification Starter Kit
2-1702-000	Strep-well HT buffer set for 10 plates
2-1703-000	Strep-well HT buffer set for 25 plates
2-1704-000	Strep-well HT buffer set for 100 plates
2-1705-010	Strep-well HT filter plates; 10 plates
2-1706-010	Strep-well HT wash plates; 10 plates
2-1707-010	Strep-well HT receiver plates; 10 plates
2-1725-010	Strep-well HT 25 Purification Plates; 10 plates
2-1725-025	Strep-well HT 25 Purification Plates; 25 plates
2-1725-100	Strep-well HT 25 Purification Plates; 100 plates
2-1750-010	Strep-well HT 50 Purification Plates; 10 plates
2-1750-025	Strep-well HT 50 Purification Plates; 25 plates
2-1750-100	Strep-well HT 50 Purification Plates; 100 plates

For research use only

Strep-tag® technology for protein purification and detection is covered by US patent 5,506,121, UK patent 2272698 and French patent 93 13 066; the tetracycline promoter based expression system is covered by US patent 5,849,576 and *Strep*-Tactin® is covered by US patent 6,103,493. Further patent applications are pending world-wide. Purchase of reagents related to these technologies from IBA provides a license for non-profit and in-house research use only. Expression or purification or other applications of above mentioned technologies for commercial use require a separate license from IBA. A license may be granted by IBA on a case-by-case basis, and is entirely at IBA's discretion. Please contact IBA for further information on licenses for commercial use. *Strep*-tag® and *Strep*-Tactin® are registered trademarks of IBA GmbH.