



Product Information

SigmaScreen™ STREPTAVIDIN HC COATED PLATES (High Capacity Plates)

<u>Product Code</u>	<u>Format</u>
S 8815	384 well; Clear
S 9315	384 well; White opaque
S 9190	384 well; Black opaque

Storage Temperature 2 to 8 °C

TECHNICAL BULLETIN

Product Description

Streptavidin HC (High Capacity) multiwell plates are prepared with a high density streptavidin coating. Streptavidin, a 60 kDa non-glycosylated protein, is isolated from *Streptomyces avidinii* and has similar biotin-binding characteristics as avidin. Streptavidin can bind 4 moles of biotin per mole of protein with high selectivity and affinity ($K_d \sim 10^{-15}$). Streptavidin has a pI of 5-6 whereas the pI of avidin is 10. The near neutral pI of streptavidin alleviates non-specific binding commonly associated with the strongly basic avidin protein.¹

The purified streptavidin is bound to the wells of the polystyrene plate via a proprietary method (patent pending). This coating technology results in strong bonding of a high density of streptavidin, in a large pore matrix, to the plate surface. This matrix provides high capacity affinity binding of biotinylated compounds and allows for unique multi-sample application potential including:

- Preparation of high capacity affinity matrices for purification of binding partners to immobilized biotinylated compound
- Purification of biotinylated compounds from heterogeneous mixtures
- Recovery of binding partners and/or biotin compounds for subsequent analysis e.g. mass spectrometry (MS) and SDS-PAGE

The plates are provided in a ready-to-use form with a lid for protection from contaminants during incubations. A preservative, chlorhexidine, is present at low levels in the wells and will not interfere with binding.

Reaction Volume

The minimum coating volume is 60 µl/well. The coating minimizes nonspecific binding.

Biotin Binding Capacity

Product Number	Minimum pmoles Biotin Binding Capacity per Well
S 8815	150
S 9315	125
S 9190	100

Capacity for biotin based on binding biotin-fluorescein. Steric hindrance will reduce the capacity of the plate when binding larger (> 5 kDa) molecules.

Precautions and Disclaimer

For Research Use Only
Not for use in diagnostic procedures

Storage/Stability

For optimal performance, the unopened product should be stored in a dry place at 2 to 8 °C. The product may be stored at room temperature for up to three months. The product should not be exposed to temperatures above 60 °C.

Refer to the Certificate of Analysis for expiration date. The Certificate of Analysis can be obtained from the Sigma-Aldrich website (www.sigma-aldrich.com).

Sample Protocols

Plate Viability

A fast validation of the plates can be accomplished as follows:

1. Dissolve 1 mg Biotin-Fluorescein (Product No. B 8889) in 4 ml Tris Buffered Saline (TBS) pH 8.0 (Product No. T 6664). Dilute the 0.25 mg/ml solution 1:30 in TBS pH 8.0 (this represents 10,000 picomoles/ml)
2. Load 60 µl (600 picomoles) of the 10,000 pmoles/ml stock per well of the plate. Incubate for 1 hour at room temperature.

- Transfer 30 μ l of the supernatant to a black 96-well non-coated plate and pipet 30 μ l of the 10,000 picomole/ml stock into additional wells on the plate to use as a control. For reference, a standard curve may be set up using biotin-fluorescein; 50-1,000 pmoles per well fall into the linear range of the curve.
- Read fluorescence (excitation at 485 nm and emission at 535 nm) comparing the biotin-fluorescein sample and control. The difference in fluorescent units represents biotin-fluorescein bound by the plate.

General

Streptavidin will bind biotin under a wide range of pH conditions, and incubation buffers may be employed at pH 4 to pH 10. Typically, physiological buffers such as Phosphate Buffered Saline (PBS) (Product No. P 3813) or TBS (e.g. T 6664) may be used with good results. An optimal negative control would be incubation of a non-biotinylated sample compared to the biotinylated sample in the experiment.

Generally, the extremely tight streptavidin/biotin binding precludes efficient recovery of the biotinylated molecules under mild conditions. Consequently, in many instances, the biotinylated molecule serves as an affinity ligand for capture of its binding partner(s). Solutions containing potential binding partners may be applied under conditions favoring the specific interaction. Incubation times and optimal concentrations of binding partners should follow the example protocols for either biotin-peptides (small molecules) or proteins (large molecules), as appropriate. Recovery of binding partner(s) may be attained by incubation with a solution chosen to disrupt the specific interaction. For example, biotinylated oligonucleotides have been used to capture complementary DNA and recovery effected by change in salt concentration.² In some cases, recovery of captured biotinylated compounds will be the objective and harsh elution conditions may be employed which provide substantial recovery. Some general elution conditions are provided in a table for consideration following the sample binding protocols.

Sample Protocol for Binding Biotin-Peptide (or other small molecules, MW < 5,000 Da)

- Prepare a solution of the biotinylated peptide in either PBS or TBS pH 7 to pH 8. An initial concentration of up to 5000 picomoles biotinylated peptide/ml may be used. For example, a peptide of 1,000 MW at 5.0 μ g/ml would allow saturation binding.

- Load up to 0.06 ml sample per well. Allow the samples to incubate 0.5 to 1 hours at 18 to 30 °C, or, as convenient, overnight (12 hours) at 2 to 8 °C. Binding is time-dependent and saturation may not be attained at the shorter incubation times.
- Wash the wells three times, 80 μ l/well with PBS or TBS containing 0.05% Tween 20 (Product No. P 3565 or T 9039) to remove unbound compounds.
- The biotinylated peptides are now selectively and firmly bound within the plate wells. The system is ready for additional incubations for capture of binding partners or direct elution/recovery of the biotin compound.

Sample Protocol for Binding Biotin-Protein (or other large molecules, MW up to 250,000 Da)

- Prepare a solution of the biotinylated protein in either PBS or TBS pH 7 to 8. An initial concentration of up to 0.25 mg biotin-protein/ml may be used. This concentration should provide saturation binding of large molecules.
- Load up to 0.06 ml sample per well. Allow the samples to incubate 1 to 2 hours at 18 to 30 °C, or, as convenient, overnight (12 hours) at 2 to 8 °C. Binding is time-dependent and saturation may not be attained at the shorter incubation times.
- Wash the wells three times, 80 μ l/well with PBS or TBS containing 0.05% Tween 20 (Product No. P 3565 or T 9039) to remove unbound compounds.
- The biotinylated proteins are now selectively and firmly bound within the plate wells. The system is ready for additional incubations for capture of binding partners or direct elution/recovery of the biotin compound.

General Elution Table

5% Formic Acid/25% Acetonitrile	Provides significant recovery of biotinylated compounds. Compatible with MS detection.
Sinapinic acid (10 ⁻¹ M) acetonitrile/H ₂ O(4:1, v/v)	Provides significant recovery of biotinylated compounds. Compatible with MALDI-TOF-MS. ³
50% Guanidine thiocyanate/formamide	Provides significant recovery of biotinylated compounds. ⁴
2M 2-mercaptoethanol (2-ME)	Provides significant recovery of biotinylated compounds. ⁵
SDS-PAGE Loading Buffer containing 2-ME (Product No. S 3401)	Provides significant recovery of many binding partners. Compatible for SDS-PAGE.
0.1% Formic Acid	Provides significant recovery of many binding partners. Compatible with MS detection.

Result Optimization

Problems may arise with poor selectivity, capacity, or recovery of molecules of interest. Recommendations for optimization are provided for each.

Nonspecific Binding

Factors that contribute to nonspecific binding are ionic interactions and hydrophobic interactions. To reduce nonspecific binding, changes to loading and wash buffers can be made. Users are encouraged to modify buffers with components as given in the compatibility table below.

Compatibility Table

Listed reagents are compatible at the indicated concentrations:

Reagents	Product #	Concentration
TWEEN® 20	P 7949	≤1.0%
SDS	L 4390	≤1.0%
Triton X-100	T 9284	≤1.0%
CHAPS	C 5849	≤1.0%
BSA	A 4503	≤1.0%
NaCl	S 3014	≤1.0 M

Low Capacity

Saturation of binding sites is time and concentration dependent. Allow up to 4 hours of incubation at 18 to 30 °C for efficient binding of large molecules such as proteins. For concentrations of molecules significantly lower than sample protocol recommendations, longer incubation times may be required. In addition, optimal results will be obtained if the biotinylated compound possesses a spacer (e.g., aminocaproic) between the biotin and the compound.⁶

Recovery of Captured Molecules

Efficient recovery of biotinylated molecules may be enhanced by careful optimization of the elution parameters. Greater recovery will be attained if the solution is allowed to incubate in the sample wells for 15 to 60 minutes. Allowing the plate to incubate at elevated temperatures (37 to 50 °C) during elution may also increase recovery. The recommended 5% formic acid/25% acetonitrile has demonstrated efficient recovery of mono-biotinylated molecules. Multi-biotinylated molecules may be recovered less efficiently.

References

1. Wilchek, M., and Bayer, E.A., Avidin-Biotin Technology. *Meth. Enzymol.*, **184** (1990).
2. Wahlberg, J., et al., General colorimetric method for DNA diagnostics allowing direct solid-phase genomic sequencing of the positive samples. *Proc. Natl. Acad. Sci. U S A.*, **87**, 6569-6573 (1990).
3. Girault, S., et al., Coupling of MALDI-TOF Mass Analysis to the Separation of Biotinylated Peptides by Magnetic Streptavidin Beads. *Analytical Chemistry*, **68**, 2122-2126 (1996).
4. Delius, H., et al., Separation of complementary strands of plasmid DNA using the biotin-avidin system and its application to heteroduplex formation and RNA/DNA hybridizations in electron microscopy. *Nucleic Acids Res.*, **13**, 5457-5469, (1985).
5. Jenne, A. and Famulok, M., Disruption of the Streptavidin Interaction with Biotinylated Nucleic Acid Probes by 2-Mercaptoethanol. *BioTechniques*, **26**, 249-254 (1999).
6. Schriemer, D.C. and Li, L., Combining Avidin-Biotin Chemistry with Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry. *Anal. Chem.*, **68**, 3382-3387 (1996).

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Plate Features

Property	96-Well Plate	384-Well Plate
Plate composition	Polystyrene	Polystyrene
Well configuration	Flat bottom/round	Flat bottom/square
Well width	6.4 mm	2.8 mm
Well depth	11 mm	11 mm
Maximum recommended working volume, per well	200 µl	60 µl

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