Product Information

SigmaScreen™ Streptavidin High-Capacity Coated Plates

S6940

Storage Temperature: –20 °C

Product Description

Streptavidin High Capacity (HC) Coated 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 ~10^{-15} M). 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. 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 multisample application potential including:

- Preparation of high-capacity affinity matrices for purification of binding partners to an immobilized biotinylated compound
- Purification of biotinylated compounds from heterogeneous mixtures
- Recovery of binding partners and/or biotin compounds for subsequent analysis. For example, 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.

Table 1. Plate Features

Property	S6940
Plate composition	Polystyrene
Well configuration	Flat bottom/round
Well width	6.4 mm
Well depth	11 mm
Maximum recommended working volume per well	200 µL

Reaction Volume: The minimum coating volume is 200 µL/well. The coating minimizes nonspecific binding. The working volume for biotinylated molecules is 50–200 µL/well.

Biotin Binding Capacity: ≥ 15 pmoles biotin per well. Capacity for biotin based on binding a biotin-fluorescein compound. Steric hindrance will reduce the capacity of the plate when binding larger (> 5 kDa) molecules.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For optimal performance, the unopened product should be stored in a dry place at 2–8 °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.

Procedures

Plate Viability

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

1. Dissolve 1 mg of 5(6)-(Biotinamidohexanoylamido) pentylthioureidylfluorescein (biotin-fluorescein, B8889) in 1 mL of DMSO. Dilute the 1.0 mg/mL solution 1:240 in TBS pH 8.0 (this represents 5,000 pmole/mL).
2. Load 200 µL (1,000 pmoles) of the 5,000 pmoles/mL solution per well of the plate. Incubate for 1 hour at room temperature.
3. Transfer 100 µL of the supernatant to a black 96-well, non-coated plate and pipette 100 µL of the 5,000 pmole/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.
4. 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 4–10. Typically, physiological buffers such as Phosphate Buffered Saline (PBS, P3813) or TBS (for example, T6664) 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 Table 2 for consideration following the sample binding protocols.

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

1. Prepare a solution of the biotinylated peptide in either PBS or TBS pH 7-8. An initial concentration of up to 2,500 pmoles biotinylated peptide/mL may be used. For example, a peptide with MW of 1,000 at 2.5 µg/mL would allow saturation binding.
2. Load up to 0.2 mL of sample per well. Allow the samples to incubate 1-2 hours at 18-30 °C, or, as convenient, overnight (12 hours) at 2-8 °C. Binding is time-dependent and saturation may not be attained at the shorter incubation times.
3. Wash the wells three times, 300 µL/well each time with PBS or TBS containing 0.05% TWEEN® 20 (P3563 or T9039) to remove unbound compounds.
4. 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.

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

1. Prepare a solution of the biotinylated protein in either PBS or TBS pH 7-8. An initial concentration of up to 0.125 mg biotin-protein/mL may be used. This concentration should provide saturation binding of large molecules.
2. Load up to 0.2 mL of sample per well. Allow the samples to incubate 2-4 hours at 18-30 °C, or, as convenient, overnight (12 hours) at 2-8 °C. Binding is time-dependent and saturation may not be attained at the shorter incubation times.
3. Wash the wells three times, 300 µL/well each time with PBS or TBS containing 0.05% TWEEN® 20 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.

Table 2. General Elution

70% Acetonitrile/5% Formic acid/ 1 mM Biotin	Provides optimal recovery; however, may not be compatible with all downstream applications such as direct reverse phase HPLC loading.
5% Formic acid/25% Acetonitrile	Provides significant recovery of biotinylated compounds. Compatible with MS detection.
Sinapinic acid (10^{-1} M) in Acetonitrile/water (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]
2 M 2-Mercaptoethanol (2-ME)	Provides significant recovery of biotinylated compounds.[5]
SDS-PAGE Loading Buffer containing 2-ME (S3401)	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 Table 3.

Compatibility Table

Table 3. Listed reagents are compatible at the indicated concentrations.

Reagents	Cat. No.	Concentration
TWEEN® 20	P7949	≤ 1.0%
SDS	L4390	≤ 1.0%
TRITON™ X-100	T9284	≤ 1.0%
CHAPS	C5849	≤ 1.0%
BSA	A4503	≤ 1.0%
NaCl	S3014	≤ 1.0 M

Low Capacity

Saturation of binding sites is time and concentration dependent. Allow up to 4 hours of incubation at 18–30 °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 (for example, 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 15-60 minutes. Allowing the plate to incubate at elevated temperatures (37-50 °C) during elution may also increase recovery. Optimal elution has been obtained by using a 70% acetonitrile/5% formic acid/1 mM biotin solution. Under most conditions, however, 5% formic acid/25% acetonitrile has demonstrated efficient recovery of mono-biotinylated molecules. Multibiotinylated 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. USA., 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).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.