SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

SigmaScreen™ Streptavidin coated plates
384-well

Catalog Numbers	**S8686**	Clear
	S8561	White opaque
	S8811	Black opaque

Storage Temperature 2-8 °C

TECHNICAL BULLETIN

Product Description

SigmaScreen Streptavidin coated plates are coated with streptavidin, a 60 kDa protein isolated from *Streptomyces avidinii*. The purified protein is bound to the wells of polystyrene microtiter plates via a proprietary coating technology. This coating technology ensures:

- high binding capacity of biotin
- high coating homogeneity
- high resistance to commonly used detergents

In addition, streptavidin-coated multiwell plates are pre-blocked for immediate use.

Binding assays of biotinylated single and double stranded DNA, peptides, proteins, and small organic molecules can be performed on streptavidin-coated multiwell plates.

Reaction Volume

Streptavidin is coated at a reaction volume of 50 µl/well. The wells are blocked at 80 µl/well.

Binding Capacity

In saturation and competitive binding assays performed on this product, binding of ≥ 4 pmoles of d-biotin per well is observed. The binding capacity for larger molecules labeled with biotin may be less than that of d-biotin due to steric hindrance associated with the specific molecule.

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 2–8 °C. Under these storage conditions, the product is stable for two years. For short-term storage of less than 3 months, the product may be stored at room temperature. Once opened, it is suggested that the product be used immediately.

Not recommended for assays at ≥60 °C.

Sample Protocols

Plate Viability Assay

To validate the viability of the streptavidin surface follow the procedure below:

1. Dissolve 1 mg of biotinylated horseradish peroxidase (P 9568) in 1 ml of phosphate buffered saline (PBS) containing 0.05% Tween-20 (P 3563). Dilute the 1 mg/ml stock 1:10,000–1:50,000 in PBS containing 0.05% Tween-20 and add 50 µl per well. As a negative control, add 50 µl of the same dilution of streptavidin-peroxidase (S 5512) to a separate set of wells.
2. Incubate the wells for 30 minutes at room temperature.
3. Wash the wells four times, 80 µl per well, with PBS containing 0.05% Tween-20.
4. After discarding the final wash, add 25 µl per well of TMB substrate (T 8665).
5. Incubate the wells for 15 minutes before reading the absorbance in a spectrophotometer. If desired, the reaction may be stopped with the addition of 0.5 M H_2SO_4 (50 µl per well). An absorbance of at least 1.5 will be observed at 655 nm for a non-acid-stopped reaction or at 450 nm for an acid-stopped reaction.

Peptide and Protein Binding

1. Prepare a solution of the biotinylated protein or peptide in either PBS or Tris buffered saline (TBS) pH 7.4. A starting concentration of 0.5–5 μg/ml should be used if the optimal concentration is not known.
2. Add up to 50 μl of the solution per well and allow the samples to incubate for 1–2 hours at a temperature within the range of 18–30 °C. Include blank and control wells as appropriate.
3. Wash the wells four times, 80 μl per well, with PBS or TBS containing 0.05% Tween-20 (P 3563 or T 9039).
4. Incubate the wells with 50 μl of an appropriately diluted primary antibody in PBS or TBS containing 0.05% Tween-20 for 30 minutes to 1 hour.
5. Wash the wells four times, 80 μl per well, with PBS or TBS containing 0.05% Tween-20.
6. Incubate the wells with 50 μl of an appropriately diluted enzyme-labeled secondary antibody in PBS or TBS containing 0.05% Tween-20 for 30 minutes to 1 hour.
7. Wash the wells four times, 80 μl per well, with PBS or TBS containing 0.05% Tween-20.
8. After addition of an appropriate substrate, the wells are ready for detection by various modes (colorimetry, chemiluminescence or fluorescence).

PCR Products

1. Prepare the biotinylated PCR product for addition onto streptavidin-coated multiwell plates by diluting the sample 1:100-1:1000 in PBS containing 0.05% Tween-20.
2. Apply 50 μl per well of the diluted sample and allow the sample to incubate for 30–60 minutes at 18–37 °C.
3. Bound PCR products are denatured by adding 0.75 M NaOH, 25 μl per well. Incubate for 5–10 minutes.
4. Wash the wells four times, 80 μl per well, with PBS containing 0.05% Tween-20 to remove the nonbiotinylated, complimentary strand of the PCR product.
5. Add 70 μl per well of a hapten-labeled oligonucleotide that is complimentary to the biotinylated strand. Use 0.02–0.2 pmole of labeled oligonucleotide per well. Hybridize in the presence of PlateHyb buffer (H 4909) or 5X SSC buffer, 0.3% Tween-20, 1% BSA. Allow the hybridization to proceed for 30–60 minutes at 37–50 °C.
6. Wash the wells four times, 80 μl per well, with PBS containing 0.05% Tween-20.
7. Add 55 μl per well of an appropriately diluted detection conjugate in PBS containing 0.05% Tween-20. Incubate for 30–60 minutes at 25-37 °C.
8. Wash the wells five to six times, 80 μl per well, with PBS containing 0.05% Tween-20.
9. Detect the bound PCR products using an appropriate substrate (see below).

Detection

There are a number of alternatives for the detection of labeled molecules. Below is a table of detection systems for two commonly used enzyme labels, horseradish peroxidase and alkaline phosphatase.

Common Enzyme Conjugates

	Peroxidase	**Alkaline Phosphatase**
Colorimetric Substrates	TMB (T 8665) ABTS (A 3219)	pNPP (A 3469) PMP (A 3344)
Chemiluminescent Substrates and Activators	Luminol/(A 8511) Iodophenol/peroxide	CDP-Star™ (C 0712)
Fluorescent Substrates	4-HPPA/(H 6386) peroxide	4-MUP (M 3168)

Troubleshooting

Optimization of ELISA Results

There are four major areas where detection can be optimized: nonspecific binding, wash conditions, antibody affinity, and conjugate concentration.

1. **Nonspecific Binding:**
   Factors that contribute to nonspecific binding are ionic interactions, hydrophobic interactions, and cross-reactivity. To reduce nonspecific binding, changes in conjugate concentrations and wash buffers can be made. Users are encouraged to modify buffers with components in the concentration ranges indicated below.

Detergents	0.05–0.1% Tween-20 (P 9416) 0.02–0.1% CHAPS (C 5070)
Salt	0.5–1.0 M NaCl (S 3014) or Na_2HPO_4 (S 3264)
Protein blockers	0.1–1% BSA (A 9647), Casein (C 3400), or Gelatin (G 7765)
Non protein blockers	1% PEG 20 (P 2263) or Polyvinylpyrrolidone (P 5288)

Wash Conditions:

To limit reversible nonspecific binding interactions, at least three wash steps are recommended.

~~3.~~2. Antibodies and Conjugates:

For optimal signal performance, the user is encouraged to use high-affinity antibodies and conjugates. Commercially obtained antibodies and conjugates should be used at the concentrations suggested by the supplier.

~~4.~~3. Miscellaneous Considerations

Due to the well geometry and surface area to volume ratio in a 384 well plate, some special circumstances arise.

- Air bubbles can be introduced easily from pipeting or washing and can create large variability in results. It is recommended to dispense liquid slowly while directing the solution against the side of the well. Air bubbles are especially likely to form when adding liquid to a dry plate. An automated liquid dispensing system may be useful for minimizing air bubbles.
- Background signals tend to be higher and sensitivity lower than what is observed in similar assays in 96 well plates.
- Mixing the contents of the wells requires vigorous shaking to obtain uniform solutions. A vortex mixer or an orbit shaker at >100 rpm may be helpful.

References

1. Wilchek, M, and Bayer, E.A. (eds.), Avidin-Biotin Technology. Meth Enzymol, **184** (1990).
2. Crowther, J.R. (ed.), ELISA: Theory and Practice. Methods in Molecular Biology, Vol **42** (Humana Press, Totowa, NJ, 1995), Product code Z36,415-0.
3. Dekonenko, A., *et al.*, A colorimetric PCR-enzyme immunoassay to identify Hantaviruses. Clin and Diagnostic Virol., **8**,113-121 (1997).
4. Morozov, I., *et al*., Mapping of functional domains in $p47^{phox}$ involved in the activation of NADPH oxidase by “peptide walking”. J. Biol. Chem., **273**, 15435-15444 (1998).
5. Hemminki, A., *et al*., Fine tuning of an anti-testosterone antibody binding site by stepwise optimisation of the CDRs. Immunotechnol., **4**, 59-69 (1998).
6. Rong, R., *et al*., Two-site immunofluorometric assay of intact salmon calcitonin with improved sensitivity. Clin. Chem., **43**, 71-75 (1997).
7. Vesanen, M., *et al.*, Detection of Herpes simplex virus DNA in cerebrospinal fluid samples using the polymerase chain reaction and microplate hybridization. J. Virol. Meth., **59,** 1-11 (1996).
8. Benotmane, A.M., *et al.*, Nonisotopic quantitative analysis of protein-DNA interactions at equilibrium. Anal. Biochem., **250,** 181-185 (1997).
9. Verhaegen, M., and Christopoulos, T.K., Quantitative polymerase chain reaction based on a dual-analyte chemiluminescence hybridization assay for target DNA and internal standard. Anal. Chem., **70,** 4120-4125 (1998).

CDP-Star™ is a trademark of Tropix, Inc.and covered under US patent 5,326,882.

PHC 11/10-1

Sigma brand products are sold through Sigma-Aldrich, Inc.
Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.