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™ ExtrAvidin® coated plates
384-well, clear

Catalog Number **E9153**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

ExtrAvidin multiwell plates are coated with ExtrAvidin, a 67 kDa protein which is a modified form of egg white avidin. ExtrAvidin combines the high specific activity and sensitivity of avidin with the low background of streptavidin. ExtrAvidin has a near neutral isoelectric point to alleviate non-specific binding interactions commonly associated with the strongly basic avidin protein. In addition, ExtrAvidin does not contain the RYD peptide sequence found in streptavidin. This is similar to the RGD cell binding domain which can cause non-specific binding of cells and/or certain receptor proteins. The purified ExtrAvidin protein is bound to the wells of polystyrene multiwell plates via a proprietary coating technology. This coating technology ensures:

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

ExtrAvidin coated multiwell plates are pre-blocked with a proprietary blocking agent for immediate use and reduced background.

Binding assays using biotinylated peptides, proteins, single and double stranded DNA, RNA, and small organic molecules can be performed on ExtrAvidin coated multiwell plates.

Reaction Volume

ExtrAvidin is coated at a minimum 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

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material 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. 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.

Refer to the Certificate of Analysis for expiration date. The Certificate of Analysis can be obtained from the Sigma-Aldrich website (www.sigmaaldrich.com). Once opened, the product should be used promptly.

Not recommended for assays at >60 °C.

Sample Protocols

Plate Performance Assay

To validate the performance of the ExtrAvidin coating follow the procedure below:

1. Dissolve 1 mg of biotinylated horseradish peroxidase, Catalog Number P9568, in 1 ml of phosphate buffered saline (PBS) containing 0.05% Tween® 20, Catalog Number P3563. Dilute the 1 mg/ml stock 1:250,000 to 1:500,000 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, Catalog Number S5512, to a separate set of wells.
2. Incubate the wells for 30 minutes at room temperature.
3. Wash the wells three times, 80 µL per well, with PBS containing 0.05% Tween 20.
4. After discarding the final wash, add 50 µL per well of TMB substrate, Catalog Number T8665.
5. Incubate the wells for 10 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 (25 µL per well). An absorbance of approximately 1.5 will be observed at 450 nm for a stopped reaction.

Peptide and Protein Binding Assay

1. Prepare a solution of the biotinylated peptide or protein in either PBS or Tris buffered saline (TBS) pH 7.4. A starting concentration of 1–10 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 three times, 80 µL per well, with PBS or TBS containing 0.05% Tween 20, Catalog Numbers P3563 or T9039.
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 three times, 80 µL per well, with PBS or TBS containing 0.05% Tween 20.
6. Incubate the wells with 80 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 three 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.

Biotinylated Oligonucleotide Binding Assay

1. Prepare the oligonucleotides for addition onto ExtrAvidin coated multiwell plates by diluting the sample in 1X Tris-EDTA buffer (prepared from 100X stock, Catalog Number T9285) to create a 40 pmole per µL stock solution of each oligo.
2. Dilute the biotinylated capture oligo to 0.2 fmole per µL in PBS + 0.05 % Tween 20, Catalog Number P3563.
3. Add 50 µL of the biotinylated capture oligo to each well. Include blank and control wells as appropriate.
4. Cover plate and allow the biotinylated capture oligo to incubate for 40 minutes at 37 °C.
5. Prepare a hybridization buffer for the oligos. Dissolve the following in 700 ml of molecular biology grade water, Catalog Number W4502:
   1 packet PBS + 0.05 % Tween 20, Cat. No. P3563
   100 g PEG MW 8000, Cat. No. P5413
   58.4 g NaCl, Cat. No. S3014
   2.5 ml Tween 20, Cat. No. P9416
   33.3 ml BSA 30% solution, Cat. No. A8327.
   Adjust volume to 1000 ml with molecular biology grade water.
6. Wash the wells three times, 80 µL per well, with PBS containing 0.05% Tween 20.
7. Dilute the complimentary oligo probe and any controls prepared in step one 1:40,000 in the hybridization buffer created in step five to yield a 1 pmol/µl solution.
8. Add 80 µL of the complimentary oligo probe and controls to all appropriate wells. In this example, a fluorescein labeled probe is used.
9. Cover the plate and incubate for 40 minutes at 37 °C.
10. Wash the wells three times, 80 µL per well, with PBS containing 0.05% Tween 20.
11. Add 80 µL of the appropriate concentration of an anti-fluorescein antibody conjugated with horse-radish peroxidase. The concentration is specific to the supplier and may need to be optimized.
12. Cover the plate and allow it to incubate for 60 minutes at room temperature (18-30 °C).
13. Wash the wells six times, 80 µL per well, with PBS containing 0.05% Tween 20.
14. Add 50 µL per well of TMB substrate, Cat. No. T8665.
15. Incubate the plate at room temperature (18-30 °C) for 10 minutes.
16. Stop the reaction by the addition of 25 µL per well of 1N H_2SO_4 to all wells.
17. Read the absorbance of the plate at 450 nm.

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 conjugates, horseradish peroxidase and alkaline phosphatase.

Detection System	Peroxidase	Alkaline Phosphatase
Colorimetric Substrates	TMB Cat. No. T8665 ABTS Cat. No. A3219	pNPP Cat. No. P7998
Chemiluminescent Substrates	Luminol Cat. No. A8511	CDP-Star® Cat. No. C0712
Fluorescent Substrates	4-HPPA Cat. No. H6386	4-MUP Cat. No. M3168

Results

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, Cat. No. P9416
	0.02–0.1% CHAPS, Cat. No. C5070
Salts 0.5–1.0 M	NaCl, Cat. No. S3014
	Na_2HPO_4, Cat. No. S3264
Protein blockers 0.1–1%	BSA, Cat. No. A9647
	Casein, Cat. No. C3400
	Gelatin, Cat. No. G7765
Non-protein blockers 1%	PEG, Cat. No. P2263
	Polyvinylpyrrolidone, Cat. No. P5288

2. **Wash Conditions:**
   To limit reversible nonspecific binding interactions, at least three wash steps are recommended.

3. **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.

References

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Troubleshooting guide

Troubleshooting Criteria	Cause	Recommendations
High background	Non-specific binding of protein in target protein solution	Decrease concentration of target protein solution. Add blocking agents, salt and/or detergents to diluent.
	Non-specific binding of detection conjugates	Add blocking agent, salt or detergents to conjugate diluents. Use higher affinity antibodies.
	Insufficient washing between incubation steps	Wash wells with 80 µL per well 3–6 times, using buffer containing at least 0.05% detergent. Increase soaking time with wash solution.
	Detection conjugates too concentrated	Dilute conjugates.
	Substrate contamination/ Degradation	Use fresh substrate reagent. Avoid repeated temperature fluctuations of pre-made substrates.
Low signal	Insufficient target protein captured	Increase concentration of target protein solution. Increase incubation time with target protein. Add detergent to buffer containing target protein to increase accessibility of biotin tag. Ensure that pH of buffer containing target protein is between 6.0-8.0 and does not contain endogenous biotin. Do not use blocking solutions which contain milk.
	Detection conjugate concentration too low	Increase concentration of conjugate solutions.
	Substrate kinetics slow	Increase incubation time with substrate. Use substrate that has been warmed to room temperature. Switch to more sensitive substrate, e.g., when using Peroxidase for detection, use TMB instead of ABTS.

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