Multiwell Plate, 96 Wells
Streptavidin Coated

Product Numbers: Z382469, Z382477, Z382485

MULTIWELL PLATE, 96 WELLS STREPTAVIDIN COATED

Technical Bulletin

INTENDED USE

For Research Use Only
Not for use in diagnostic procedures.

PRODUCT DESCRIPTION

Streptavidin multiwell plates are coated with streptavidin, a 60 KD 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
- low leaching of streptavidin (< 6 ng per well)
- high resistance to commonly used detergents

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

High capacity 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 100 µl/well. The wells are blocked at 200 µl/well.

BINDING CAPACITY

In saturation and competitive binding assays performed on this product, binding of ≥ 25 pmoles of d-biotin per well is observed. The binding capacity of larger molecules labeled with biotin will possibly be less than that of biotin via steric factors associated with the specific molecule.

STORAGE AND HANDLING CONDITIONS:

For optimal performance, the unopened product should be stored in a dry place at 2–30°C. Under these storage conditions, the product is stable for two years. Once opened, it is suggested that the product be used immediately.

Not recommended for assays at > 60°C.

SAMPLE PROTOCOLS FOR THE USE OF STREPTAVIDIN COATED MULTIWELL PLATES:

PLATE VIABILITY ASSAY

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

1. Using catalog item P 9568 (Biotinylated HRP), dissolve 1 mg in 1 ml of PBS + 0.05% Tween-20 (P 3563).
   Dilute the 1 mg/ml stock 1:50,000–1:100,000 in PBS + 0.05% Tween-20 and add 100 µl per well. As a negative control, add 100 µl of a similar dilution of streptavidin-peroxidase (S 5512) to a separate set of wells.
2. At room temperature, incubate the wells for 30 minutes.

3. Wash the wells three times, 300 µl per well, with PBS + 0.05% Tween-20.
4. After discarding the final wash, add 100 µl per well of TMB substrate (T 8665).
5. Incubate the wells for 15 minutes before reading in a spectrophotometer. If desired, the reaction may be stopped with the addition of 0.5M H_2SO_4 (50 µl per well). An absorbance of at least 1.5 will be observed at 655 nm for a non-stopped reaction or 450 nm for a stopped reaction.

PEPTIDE AND PROTEIN BINDING

1. Prepare a solution of the biotinylated protein or peptide in 0.05M bicarbonate buffer pH 9.6, PBS pH 7.4 or TBS pH 7.5. A starting concentration of 1–10 µg/ml should be used if the optimal concentration is not known.
2. Add up to 100 µl of the solution per well and allow the samples to incubate for 1–2 hours at a temperature range of 18–30°C.
3. Wash the wells three times, 300 µl per well, with PBS or TBS + 0.05% Tween-20.
4. Incubate the wells with 100 µl of an appropriately diluted primary antibody in PBS or TBS + 0.05% Tween-20 for 30 minutes to 1 hour.
5. Wash the wells three times, 300 µl per well, with PBS or TBS + 0.05% Tween-20.
6. Incubate the wells with 100 µl of an appropriately diluted enzyme labeled secondary antibody in PBS or TBS + 0.05% Tween-20 for 30 minutes to 1 hour.
7. Wash the wells three times, 300 µl per well, with PBS or TBS + 0.05% Tween-20.
8. After addition of an appropriate substrate, the wells are ready for detection in various modes (colorimetry, chemiluminescence or fluorescence).

PCR PRODUCTS

1. Prepare the biotinylated PCR product for addition onto streptavidin coated multiwell plates by diluting the reaction 1:10–1:50 in PBS + 0.05% Tween-20.
2. Apply 100 µl per well of the diluted product and allow the sample to incubate for 30–60 minutes at 25–37°C.
3. Bound PCR products are denatured by adding 100 µl per well of 0.5M NaOH. Incubate for 5–10 minutes.
4. Wash the wells three times, 300 µl per well, with PBS + 0.05% Tween-20 to remove the nonbiotinylated, complimentary strand of the PCR product.
5. Add 200 µl per well of a hapten labeled oligonucleotide which is complimentary to the biotinylated strand. Use 0.05–0.5 pmole of labeled oligonucleotide per well. Hybridize in the presence of 5X SSC, 0.3% Tween-20, 1% BSA. Allow the hybridization to proceed for 30–60 minutes at 37–50°C.
6. Wash the wells three times, 300 µl per well, with PBS + 0.05% Tween-20.
7. Add 100 µl per well of an appropriately diluted detection conjugate in PBS + 0.05% Tween-20. Incubate for 30–60 minutes at RT.
8. Wash the wells five to six times, 300 µl per well, with PBS + 0.05% Tween-20.
9. Proceed to the use of the specific substrate for detection.

DETECTION

There exists a number of alternatives for the detection of molecules labeled with common haptens. Below is a table of indirect detection systems for two haptens, namely biotin and fluorescein.

	Common Colorimetric Substrates	Common Chemiluminescent Substrates and Activators
Peroxidase	TMB, ABTS	Luminol/ Iodophenol/peroxide
Alkaline phosphatase	pNPP	dioxetanes
Acridinium ester		$NaOH/H_2O_2$
Beta-galactosidase		dioxetanes

OPTIMIZATION OF ELISA RESULTS

There are four major areas where detection results can be optimized: nonspecific binding, antibody affinity, wash conditions, 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 ranges of the various conditions stated below.

Detergents	0.05–0.1% Tween-20 0.002–0.05% Tween-65
Salt	0.5–1.0 M NaCl or Na_2HPO_4
Protein blockers	0.1–1% BSA or casein
Non protein blockers	1% PEG 20 or Polyvinylpyrrolidone

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.

RELATED PRODUCTS

T 8665 3,3',5,5'-Tetramethylbenzidine (TMB) Liquid Substrate System
A 9941 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) tablets
P 4922 Phosphate-citrate buffer tablets with Sodium Perborate capsules
N 7653 p-Nitrophenyl Phosphate (pNPP) Liquid Substrate System
P 3813 Phosphate Buffered Saline, pH 7.4
P 3688 Phosphate Buffered Saline with BSA, pH 7.4
P 3563 Phosphate Buffered Saline with Tween-20, pH 7.4
T 6664 Tris Buffered Saline, pH 8.0
T 9039 Tris Buffered Saline with Tween-20, pH 8.0
P 1318 Phosphatase, Alkaline-biotinamidocaproyl labeled
P 9568 Peroxidase-biotinamidocaproyl labeled
S 5512 Streptavidin-peroxidase labeled
S 6639 20X SSC Buffer concentrate
A 8327 30% BSA solution
P 9416 Polyoxyethylenesorbitan monolaurate (Tween-20)
BK-200 Cleavable Biotinylation Kit
BK-101 Immunoprobe Biotinylation Kit
B-TAG Biotin Tag Micro Biotinylation Kit
OLB-16-DDU BioProbe 3'Oligonucleotide Labeling Kit with Bio-16-ddUTP

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