For Research Use Only Not for Diagnostic Use

Express Capture Streptavidin Coated Plates

A rapid screening tool from Express Bio

Catalog Numbers:

EX8000-C96 (Clear 96 well plate)
EX8000-B96 (Black 96 well plate)
EX8000-W96 (White 96 well plate)
EX8000-C384 (Clear 384 well plate)
EX8000-B384 (Black 384 well plate)
EX8000-W384 (White 384 well plate)

Product Description

The product provides a protocol for the screening of biotinylated biomolecules that is reliable, fast, and has high throughput capabilities. Streptavidin is a protein isolated from *Streptomyces avidinii* that has high affinity binding for biotin. Like its namesake avidin, streptavidin binds 4 moles of biotin per mole of protein with a high affinity virtually unmatched in nature (Kd~10^{-15}). Streptavidin lacks the carbohydrate side chains present on avidin and has an isoelectric point nearer to neutrality where most useful biological interactions occur (pI of 5-6 vs 10 for avidin). As a result, streptavidin frequently exhibits lower levels of non-specific binding than avidin when the proteins are used in applications relying upon the formation of avidin/biotin complexes. This product may be used with biotinylated peptides, antigens, antibodies, DNA, and oligonucleotides for various uses such as immunoassays and DNA/RNA quantitation.

Components

One streptavidin coated microassay plate: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant/humidity indicator card. The plate should be stored at refrigerated temperature (4 °C). Unused strips should be stored resealed in the foil pouch. The product is stable for one year when stored properly.

Materials and Equipment Required But Not Provided

Biotinylated biomolecule
Detection enzyme-conjugate
Enzyme substrate
Pipettes and Tips (Multi-channel)
Wash bottle or machine
Microtiter plate reader

Procedures

I. Coupling of Biotinylated Biomolecule

Buffers:
Oligonucleotides:

5 x SSCT, pH=7.0, 750 mM NaCl, and 75 mM Sodium Citrate) containing 0.05% (v/v) TWEEN® 20

2 x SSCT, pH=7.0, 300 mM NaCl, and 30 mM Sodium Citrate) containing 0.05% (v/v) TWEEN® 20

Proteins and Peptides:

PBST, pH=7.2, Phosphate Buffered Saline containing 0.05% (v/v) TWEEN® 20

Recommended Concentrations:

Oligonucleotides: 0.5µM – 0.01µM diluted in 5 x SSCT buffer

Proteins: 5ug/ml – 0.05ug/ml diluted in a PBST buffer

Peptides: 1ug/ml– 1ng/ml diluted in a PBST buffer

Protocol:

1. Using a wash bottle or automated washing equipment add 250-300 ul of diluted wash buffer to each well. (5X SSCT buffer for oligonucleotides, PBST buffer for proteins or peptides) Aspirate or shake out and turn plate upside down and blot on paper towel to remove all liquid. Repeat the wash procedure two times (for a total of three washes).

2. Prepare the biotinylated biomolecule in the appropriate buffer.

3. Add 100ul of the solution to the microtiter plate.

4. Incubate for one hour at room temperature. Agitation on a rotary mixer can be used (100rpm).

5. Remove the liquid and wash using the wash protocol in step one with 2X SSCT buffer for oligonucleotides or PBST for proteins and peptides.

II. Detection of Biomolecule

Many methods can be utilized to detect the captured biotinylated biomolecule. It will usually be a ligand conjugated to an enzyme that will attach to the biomolecule. The following example illustrates the detection of a protein.

1. Make a 2-fold dilution of an enzyme conjugated antibody against the protein in PBST buffer. Add 100ul of each dilution to the microtiter plate that has been coupled with the biotinylated protein.

2. Incubate at room temperature for 60 minutes.

3. Aspirate the wells and wash three times with PBST buffer as described above.

4. Add 100ul of enzyme substrate and incubate at room temperature for 15 minutes.

5. Add 100ul of appropriate reagent to stop the reaction.

6. Read the plate on a microplate reader at the appropriate wavelength for the stopped substrate.

Critical Steps

1. Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semi-automated or automated equipment set to deliver a volume to completely fill each well (250-300 ul) is recommended. Complete removal of the wash buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.

2. High backgrounds can occur from inadequate washing or from too highly concentrated detection materials. Careful titration of ligands will result in optimal assay.

3. Low signals can also occur from inadequate titration of detection materials.

4. All reagents must be at room temperature before running the assay. Remove only the volume of reagents that is needed. Do not pour reagents back into vials as reagent contamination may occur.

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