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Streptavidin Coated 96-well Microtiter Plates

CAT#: CLSTAN-5P

INTRODUCTION:

Streptavidin is a 52.8 KDa protein originally isolated from the bacterium Streptomyces avidinii that naturally forms a homo-tetramer. Each of the four subunits of streptavidin contain a single binding site for biotin. Streptavidin has an extremely high affinity for biotin, having a dissociation constant (Kd) on the order of ~10−14 mol/L; this makes it one of the strongest non-covalent interactions known in nature. Additionally, Streptavidin has no carbohydrate groups and a near neutral isoelectric point, resulting in low nonspecific interactions. Taken together, the high affinity and low background interactions of streptavidin and biotin make it an ideal system for use in biological assays.

Cedarlane's Streptavidin Coated Microtiter Plates are coated with highly purified streptavidin. After coating, the plates are blocked to ensure low non-specific binding and stability as well as allowing for direct use. The plates are useful for capturing biotinylated proteins, peptides and DNA and can be used in various applications including ELISA and measure of protein-protein interactions.

STORAGE/STABILITY:

The microplates, if unopened, are stable refrigerated until the expiration date printed on the label. If opened, store in closed pouch with desiccant and use within 2- 4 weeks.

SPECIFICATIONS:

Components
Five (5) individually pouched 96-well plates, configured in 12 removable 8-well strips.

Coating
Purified Streptavidin is coated using 100 µl/well. Nominal binding capacity is ~10 pmol biotin/well. The strips are blocked with 200 µl/well to ensure low non-specific binding and long-term stability.

Sensitivity
Biotinylated IgG was detected at concentrations significantly above background in an ELISA format using anti-rabbit or mouse IgG-HRP (at 0.1 µg/ml) as detector and TMB as substrate (see Figure 1 below), as follows:

Rabbit IgG – 0.156 ng/well
Mouse IgG – 0.234 ng/well

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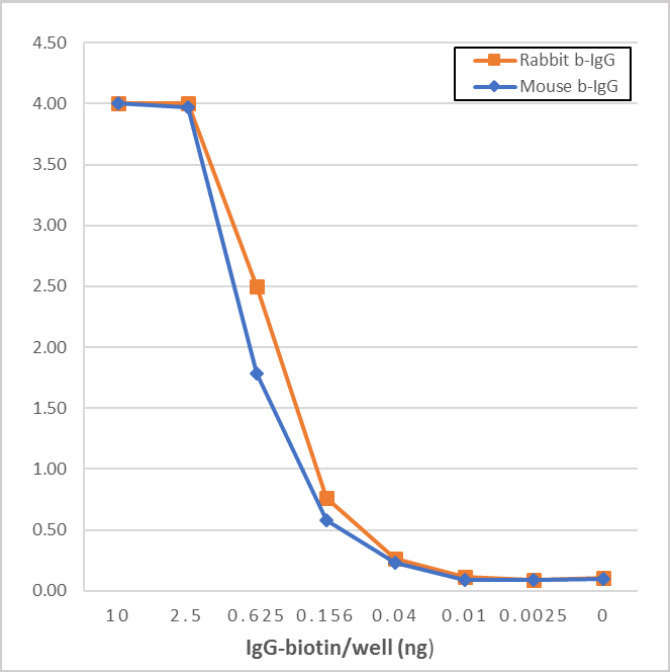

Figure 1. Capture of biotinylated rabbit and mouse IgG.

APPLICATION:

Example ELISA Procedure:

A. Materials Required

- Wash Buffer / Assay buffer: 1x phosphate-buffered saline, 0.05% Tween®-20, pH 7.4 or alternative buffer (assay dependent).
- Biotinylated capture antibody to antigen of interest diluted in wash buffer.
- Antigen adjusted to appropriate concentration with wash buffer.
- Enzyme-labeled (HRP or ALP) primary antibody, or primary antibody + enzyme-labeled secondary antibody diluted in wash buffer.
- Appropriate enzyme substrate (TMB or Phosphatase); stop solution (1M H_2SO_4).

B. Method

1. Add 100µL of the biotinylated capture antibody to each well and incubate for 1 hour at 37°C.
2. Wash each well five times with 300µL of Wash Buffer. Make a serial dilution of the antigen and add 100µL to each well. Incubate plate for 1 hour at 37°C.
3. Wash each well five times with 300µL of Wash Buffer. Add 100µL of the primary antibody (unconjugated) or primary antibody-HRP conjugate to each well and incubate plate for 1 hour at 37°C.
4. Wash each well five times with 300µL of Wash Buffer.
5. Proceed to TMB step in the case of antibody-HRP conjugate or add 100µL of the HRP-labeled secondary antibody to each well. Incubate plate for 1 hour at 37°C. Wash each well five times with 300µL of Wash Buffer.
6. Add 100µL of TMB and incubate at RT x 15-30 min.
7. Add 100µL of Stop Solution, read absorbance at 450nm and process data.