Product Manual

Streptavidin ELISA Kit

Catalog Numbers

AKR-5186	96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Streptavidin is a tetrameric 60 kDa bacterial protein isolated from *Streptomyces avidinii* providing 4 high-affinity biotin binding sites. Streptavidin-biotin binding is one of the strongest non-covalent bonds found in nature, with a femtomolar dissociation constant (Kd ~10^{-15}). This binding affinity is around 10^3-10^6 times higher than the antigen-antibody interactions. The streptavidin-biotin complex has high thermal stability, it also possesses strong resistance to denaturants, organic solvents, proteolytic enzymes, detergents, and extreme pH. Because of its binding characteristics, streptavidin is commonly employed for immunotechniques requiring signal amplification using biotinylated reagents.

Cell Biolabs' Streptavidin ELISA Kit is an enzyme immunoassay developed for detection and quantitation of Streptavidin or Streptavidin fusion proteins in cell or tissue samples. The kit has a detection sensitivity limit of 15 pg/mL Streptavidin. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and Streptavidin samples.

Assay Principle

The Streptavidin protein standards, or unknown Streptavidin or Streptavidin fusion samples, are first added to a biotinylated conjugate coated plate. After incubation, an anti-Streptavidin polyclonal antibody is added, followed by an HRP conjugated secondary antibody. The Streptavidin content in unknown samples is determined by comparing with a standard curve that is prepared from Streptavidin protein standards.

Related Products

1. AKR-110: Rapid GST Inclusion Body Solubilization and Renaturation Kit
2. AKR-121: GFP ELISA Kit
3. AKR-130: His Tag Protein ELISA Kit
4. AKR-5185: GST ELISA Kit
5. AKR-5187: Avidin ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Biotin Conjugate Coated Plate (Part No. 51861B): One strip well 96-well plate.
2. Anti-Streptavidin Antibody (1000X) (Part No. 51862C): One 20 µL vial of anti-Streptavidin Rabbit IgG.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 231009): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Streptavidin Standard (Part No. 51863D): One 100 µL vial of 0.25 µg/mL Streptavidin from *Streptomyces avidinii* in PBS containing BSA.

Materials Not Supplied

1. Streptavidin or Streptavidin Fusion Protein Sample: cell or tissue lysate
2. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the recombinant Streptavidin Standard at -20ºC and avoid freeze/thaw. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Anti-Streptavidin Antibody and HRP-Conjugated Secondary Antibody: Immediately before use dilute the anti-streptavidin antibody 1:1000 and HRP-conjugated secondary antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Streptavidin Standard

Prepare a dilution series of Streptavidin standards in the concentration range of 0 pg/mL to 1000 pg/mL in Assay Diluent (Table 1).

Standard Tubes	0.25 µg/mL Streptavidin Standard (µL)	Assay Diluent (µL)	Streptavidin (pg/mL)
1	4	996	1000
2	500 of Tube #1	500	500
3	500 of Tube #2	500	250
4	500 of Tube #3	500	125
5	500 of Tube #4	500	62.5
6	500 of Tube #5	500	31.2
7	500 of Tube #6	500	15.6
8	0	500	0

Table 1. Preparation of samples for Streptavidin Standard Curve

Assay Protocol

1. Prepare cell or tissue lysates containing Streptavidin or Streptavidin fusion protein.
2. Add 100 µL of Streptavidin sample or Streptavidin standard to the Biotin Conjugate Coated Plate. Each Streptavidin sample, Streptavidin standard and blank should be assayed in duplicate.
3. Incubate at room temperature for 2 hours on an orbital shaker.
4. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Anti-Streptavidin Antibody to each well.
6. Incubate at room temperature for 2 hours on an orbital shaker.
7. Wash the strip wells 3 times according to step 4 above.
8. Add 100 µL of the diluted HRP-Conjugated Secondary Antibody to all wells.
9. Incubate at room temperature for 1 hour on an orbital shaker.
10. Wash the strip wells 3 times according to step 4 above. Proceed immediately to the next step.
11. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
12. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
13. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the Streptavidin ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

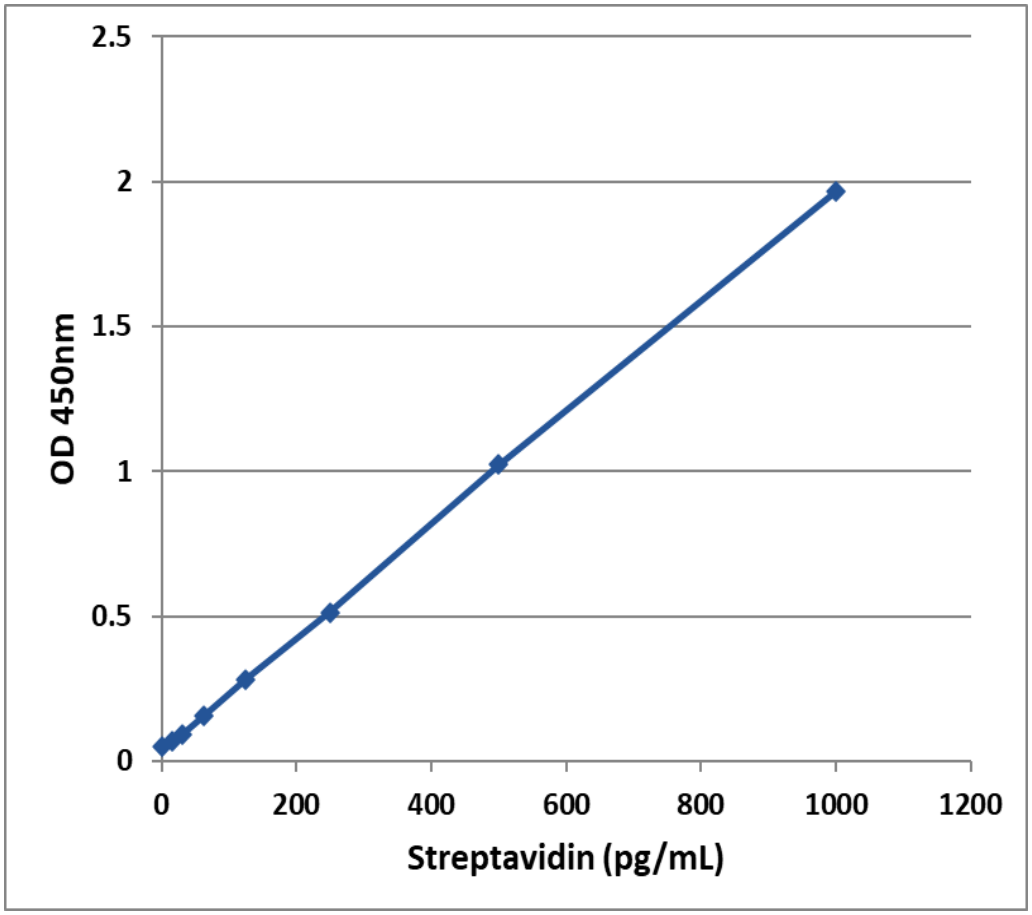

Figure 1: Streptavidin ELISA Standard Curve.

References

1. Simons, P.C. and VanderJagt, D.L. (1977). *Anal Biochem* **82**:334-41.
2. Smith DB, Johnson KS. (1988) *Gene.* **67**:31–40.
3. Frangioni, J.V. and Neel, B.G. (1993). *Anal Biochem* **210**:179-87.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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