Product Manual

Avidin ELISA Kit

Catalog Numbers

AKR-5187	96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Avidin is a basic tetrameric glycoprotein (66-69 kDa) composed of four identical subunits, each binds to biotin with high specificity and affinity (K_d ~10^{-15} M). Avidin is originally derived from the eggs of aves, reptiles and amphibians. Avidin-biotin interaction is considered one of the most specific and stable non-covalent interactions, which is about 10^3 to 10^6 times higher than an antigen-antibody interaction. Similar to Streptavidin, the Avidin-biotin complex has high thermal stability, and it possesses strong resistance to denaturants, organic solvents, proteolytic enzymes, detergents, and extreme pH. Because of its binding characteristics, Avidin is commonly employed for immunotechniques requiring signal amplification using biotinylated reagents.

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

Assay Principle

The Avidin protein standards, or unknown Avidin or Avidin fusion samples, are first added to a biotinylated conjugate coated plate. After incubation, an anti-Avidin polyclonal antibody is added, followed by an HRP conjugated secondary antibody. The Avidin content in unknown samples is determined by comparing with a standard curve that is prepared from Avidin 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-5186: Streptavidin 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-Avidin Antibody (1000X) (Part No. 51871C): One 20 µL vial of anti-Avidin 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. Avidin Standard (Part No. 51872D): One 100 µL vial of 1 µg/mL Avidin from egg white in PBS containing BSA.

Materials Not Supplied

1. Avidin or Avidin 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 Avidin 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- Avidin Antibody and HRP-Conjugated Secondary Antibody: Immediately before use dilute the anti-Avidin antibody 1:1000 and HRP-conjugated secondary antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Avidin Standard

Prepare a dilution series of Avidin standards in the concentration range of 0 pg/mL to 4000 pg/mL in Assay Diluent (Table 1).

Standard Tubes	1 µg/mL Avidin Standard (µL)	Assay Diluent (µL)	Avidin (pg/mL)
1	4	996	4000
2	500 of Tube #1	500	2000
3	500 of Tube #2	500	1000
4	500 of Tube #3	500	500
5	500 of Tube #4	500	250
6	500 of Tube #5	500	125
7	500 of Tube #6	500	62.5
8	0	500	0

Table 1. Preparation of samples for Avidin Standard Curve

Assay Protocol

1. Prepare cell or tissue lysates containing Avidin or Avidin fusion protein.
2. Add 100 µL of Avidin sample or Avidin standard to the Biotin Conjugate Coated Plate. Each Avidin sample, Avidin 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-Avidin 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 Avidin ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

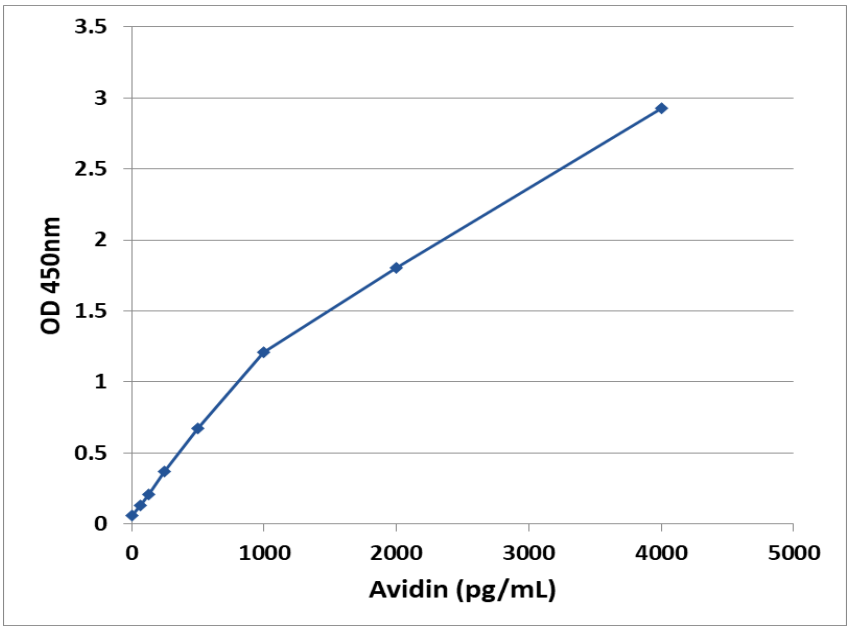

Figure 1: Avidin ELISA Standard Curve.

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.
4. Yao Z, et al. (1998) *Journal of the National Cancer Institute.* **90**:25–29.
5. Rosano C, Arosio P, Bolognesi M. (1999) *Biomol Eng.* **16**:5–12.
6. Livnah O, et al. (1993) *Proceedings of the National Academy of Sciences of the United States of America.* **90**:5076–5080.
7. Pazy Y, et al. (2003) *Journal of Bacteriology.* **185**:4050–4056.

Warranty

Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com