EAGLE BIOSCIENCES

High Sensitivity Streptavidin Coated 96 Well Microplate (Clear)

Catalog Number:
STP00-K05 (5 x 96 wells)
STP00-K20 (20 x 96 wells)
For Research Use Only (RUO). Not for use in clinical, diagnostic or therapeutic procedures.
v. 1.0

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
WWW.EAGLEBIO.COM

I. Intended Use

The Eagle Biosciences High Sensitivity Streptavidin Coated 96 Well Microplate (Clear) is a pre-blocked, clear 12 x 8-well, strip microplate that can be used for specific capture of biotin and biotinylated molecules. The High Sensitivity Streptavidin Coated 96 Well Microplate is used as solid support for the binding of biotinylated molecules in a variety of assays for the measurement of drugs, peptides, proteins, DNA/RNA (nucleic acids), and a host of other molecules.

The High Sensitivity Streptavidin Coated 96 Well Microplates are not intended for capture ***and release*** of biotinylated molecules as the streptavidin is passively, not covalently, bound to the microplates. Any process used to release the biotinylated antibody from the streptavidin will, in all likelihood, release the streptavidin from the microplate.

These microplates are intended For RESEARCH USE ONLY (RUO) and are not to be used for Diagnostic or Therapeutic Procedures.

II. Summary and Explanation: Streptavidin Microplate

Streptavidin (MW ~55,000 Daltons) is a tetrameric protein isolated from *Streptomyces avidinii*, which specifically binds biotin (244 Daltons). The binding between streptavidin and biotin is one of the strongest non-covalent bindings known at (K_d) of ~10^{-14} mol/L (biotin-streptavidin complex).

Unlike avidin, streptavidin in non-glycosylated, and is mildly acidic (pI~5.5), whereas avidin is basic (pI~10.5). Because streptavidin lacks glycosylated moieties and has a lower pI than avidin, streptavidin has considerably less non-specific binding which results in less background signal in many tests. These characteristics make streptavidin an ideal choice for a variety of applications including studies with cells, nucleic acids, drugs, small/large molecules, peptides, and proteins.

III. Materials Provided

Store all microplates at 2- 8°C and protect from light for long periods. Do not freeze the High Sensitivity Streptavidin Coated 96 Well Microplates. Expiration dates and lot numbers are printed on the individual component labels.

Description	Size
96-well Streptavidin 12 x 8-well, strip, pre-blocked	5 plates (STP00-K05) 20 plates (STP00-K20)

IV. Materials Required but not Supplied

1. Single or multi-channel precision pipettes with disposable tips: 10-100µl and 50-200µl
2. Pipettes: 1 mL, 5 mL, 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Microtiter plate reader (450 nm ± 2nm)
6. Automatic microtiter plate washer or squirt bottle.
7. Highly purified water.
8. Disposable gloves.
9. Absorbent paper.
10. Graduated Cylinder
11. Additional Components Available from Eagle Biosciences:
    a. Dilution Buffer: DIL-55, 55 mL, ready to use
    b. 20x PBS Buffer: PBS-55, 55 mL, concentrate
    c. 20x Wash Buffer: WBU-250 mL, 250 mL, concentrate
    d. TMB Substrate: TMB-55, 55 mL, ready to use
    e. Stop Solution: STP-250, 250 mL ready to use

V. Microplate Preparation

Plate Strips	Remove appropriate number of strips needed for testing and return unused strips to foil pouch. Seal pouch with strips and desiccant and store at 2-8°C. Upon completion of testing, save microplate frame for future testing with stored strips. It is advisable to label the top of strip to keep proper orientation of the strip and testing pattern. *Note: In some assays increased sensitivity has been demonstrated when the microplate is pre-washed with Wash Buffer (Eagle WBU-250 or similar) (To be determined by user.)*

VI. Typical Assay Procedure

1. Equilibrate microplate strip to room temperature.
2. Add 100 µl of the appropriately diluted biotinylated sample (suggest range 10-400 ng/mL) to each well of the 8-well strip. (Dilution Buffer: Eagle Biosciences DIL-55 or similar for protein and TE Buffer for nucleic acids)

   Note: Increased sensitivity may be achieved by pre-washing the well with 1x Wash Buffer 4x. (Eagle Wash Buffer WBU-250 or similar)

3. Carefully cover wells with new adhesive film. Mix on plate shaker (300-600 rpm) for 1 hour at room temperature (18-25°C).
4. Carefully remove adhesive film, empty well contents and wash four times using 300µl per well of Wash Buffer (Eagle Biosciences WBU-250 or similar).
5. Add 100 µL of labeled secondary antibody (titered properly) or other detection methods.
6. Carefully cover wells with new adhesive film. Mix on plate shaker (300-600 rpm) for 1 hour at room temperature (18-25°C).
7. Add 100 µl of TMB Substrate (or appropriate substrate) to each well. Incubate 15 minutes (0.5 - 30 minutes depending upon intensity of signal) at room temperature (18–25°C). (Eagle Biosciences TMB-55)
8. Add 100 µl of Stop Solution which changes blue color in wells to yellow. (Eagle STP-250 or similar)
9. Measure absorbance using plate reader at wavelength 450 nm (or appropriate wavelength)

VII. Troubleshooting

1. **Weak or No Signal**
   a. Low or no biotinylated molecule present
   b. Interference of buffers (Sample, Dilution, or Wash)
      1. Harsh Conditions such as high ionic strength, detergent concentration, strong acid or base, organic solvents.
   c. Instrument Reading Settings are inappropriate
   d. Incapability of reagents
   e. Signal Substrate expired or contaminated

2. **High Background or Signal**
   a. Dilute sample further
   b. Detection antibody or detection system not properly titered (reduce concentration of antibody or detector)
   c. Washing procedure or buffer not effective
   d. Non-specific binding of contaminates
   e. Signal Substrate expired or contaminated

VIII. Product Characteristics

1. Limit of Detection*: 2 ng/mL (biotinylated Mouse IgG)
2. Range*: 2-2,500 ng/mL (biotinylated Mouse IgG)
3. Precision: <5% between individual wells. <8% between plates
4. Specificity: Biotin or Biotinylated molecules

*As determined by colorimetric assay using biotinylated Mouse IgG and goat anti-mouse IgG-HRP for detection.

IX. References

1) Green N.M. (1963). Avidin. 3. The Nature of the Biotin-Binding Site. *Biochem J.* **89:**599–609.
2) Green, N. M. 1975. Avidin. In Advances in Protein Chemistry. M. C. Anson and J. T. Edsell, editors. Academic Press, New York. 85-133.
3) Wilchek, M., and E. A. Bayer. 1990. Avidin-biotin technology. In Methods in Enzymology. Vol. 184. Academic Press, New York. 5-51.

X. Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this High Sensitivity Streptavidin Coated 96 Well Microplate insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.