

## ORDERING INFORMATION & SPECIFICATIONS

**Coating Compound:** Streptavidin

**Concentration:** 4 µg/mL

**Specificity:** Biotinylated Molecules

**Coating Volume:** 200 µL/well

**Blocking Volume:** 330µL/well

### Typical Working Concentrations:

Biotinylated Antibodies: 0.1-1.0 µg/well

Biotinylated Oligonucleotides: 1.0-5.0 pmol/well

## INTENDED USE

EvenCoat™ Streptavidin Microplates are clear, pre-blocked 96-well polystyrene microplates specific for biotin and biotin-conjugated compounds. These plates may be used as a solid support for assays that utilize a biotinylated molecule or otherwise require the binding of biotin. Examples include immunoassays (biotinylated antibody or antigen)(1, 2), the measurement of DNA or RNA (biotinylated oligonucleotide) (3-6), transcription factor or DNA binding proteins assays (biotinylated DNA)(7, 8), and kinase assays (biotinylated peptide)(9).

## STABILITY AND STORAGE

Unopened plates may be stored at 2-8 °C. Upon opening, return unused wells to the foil pouch containing the desiccant pack, reseal along the entire edge of zip-seal. Plates may then be stored for up to 1 month at 2-8 °C.

## PROTOCOL FOR HYBRIDOMA SCREENING

1. Add 100 µL of biotinylated antigen to each well. Cover with an adhesive strip and incubate for 30 minutes at room temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (350 µL/well) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
3. Add 100 µL of hybridoma supernate, standard, or media blank to respective wells. Cover with an adhesive strip and incubate for 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 µL of labeled secondary antibody to each well. Cover with an adhesive strip and incubate for 1 hour at room temperature.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of an appropriate substrate to each well. Cover with an adhesive strip. Incubation times may vary depending on the labeled secondary antibody and the type of substrate used.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density immediately, using a microplate reader.

## REFERENCES

1. Clavijo, A. *et al.* (1998) *Vet. Microbiol.* **60**:155.
2. Ternyck, T. *et al.* (1990) *Methods Enzymol.* **184**:469.
3. Whitby, K. *et al.* (1995) *J. Virol. Methods* **51**:75.
4. Kohler, T. *et al.* (1998) *Biotechniques* **25**:80.
5. Xiao, L. *et al.* (1996) *J. Immunol. Methods* **199**:139.
6. Holmstrom, *et al.* (1993) *Anal. Biochem.* **209**:278.
7. McKay, I.A. *et al.* (1998) *Anal. Biochem.* **265**:28.
8. Benotmane, A.M. *et al.* (1997) *Anal. Biochem.* **250**:181.
9. Koch, T. *et al.* (2000) *Bioconjugate Chem.* **11**:474.