

ORDERING INFORMATION & SPECIFICATIONS

Coating Antibody: Goat Anti-Mouse IgG

Specificity: mouse IgG Fc Region

Coating Volume: 100 µL/well

Blocking Volume: 300 µL/well

Suggested Mouse IgG Concentration:

1.0-5.0 µg/mL
(0.1-0.5 µg/well)

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 SANDWICH ELISA USING DUOSET DEVELOPMENT KITS

Note: *EvenCoat™ Microplates may be used with most DuoSet® ELISA Development Kits.*

Reconstitute DuoSet® reagents, prepare working concentrations, and prepare ancillary reagents (except Block Buffer, which is not required when using EvenCoat™ Microplates) as instructed in the DuoSet® product insert.

1. Add 100 µL of diluted capture antibody to each well. Cover with an adhesive strip and incubate for 1 hour 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 (400 µL) using a squirt bottle, multi-channel pipette, 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 sample or standard in reagent diluent (or an appropriate diluent) to each well. Cover with an adhesive strip and incubate for 1.5-2.0 hours at room temperature.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 µL of detection antibody, diluted in reagent diluent, to each well. Cover with an adhesive strip and incubate for 1.5-2.0 hours at room temperature.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of the working dilution of Streptavidin-HRP to each well. Cover with an adhesive strip and incubate for 20 minutes at room temperature.
Note: *Streptavidin-HRP concentration may have to be adjusted for optimal signal generation.*
8. Repeat the aspiration/wash as in step 2.
9. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. **Protect from light.**
10. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

INTENDED USE

EvenCoat™ goat anti-mouse IgG microplates are clear, pre-blocked 96-well polystyrene microplates coated with goat-derived antibody specific for the Fc region of mouse IgG. These plates may be used as a solid support for most sandwich ELISAs utilizing a mouse IgG capture antibody and a non-mouse IgG detection antibody. Other applications include competitive ELISA, IgG isotyping, and hybridoma screening/selection. An example protocol for a sandwich ELISA is described below.

RELATED PRODUCTS

- DuoSet® ELISA Development Kits (See website).
- Matched Antibody Pairs (See website).
- Streptavidin-HRP (R&D Systems®, Catalog # DY998).
- Substrate Solution (R&D Systems®, Catalog # DY999).
- Wash Buffer (R&D Systems®, Catalog # WA126).