

## **SECONDARY ANTIBODIES COATED SURFACE: GOAT ANTI MOUSE IgG Fc $\gamma$ (Subclasses 1+2a+2b+3)**

The Biomat product is a 96 well coated microplate with goat anti mouse IgG Fc $\gamma$  (subclasses 1+2a+2b+3) and a protein to block non-specific binding sites and to maintain stable activity.

Affinity purified goat anti mouse IgG specifically binds the Fc region of mouse immunoglobulin subclasses 1,2a,2b and 3, with minimal cross-reaction to human, bovine and rabbit serum proteins.

These plates may be used as solid support for most sandwich ELISAs utilizing a mouse IgG capture and a non mouse IgG detection antibody. Other applications include competitive ELISA, IgG isotyping and hybridoma screening/selection.

These plates are ideal for binding assays when available antibodies are in low quantities or they denature and become inactive upon direct adsorption to polystyrene plates.

### ***Features of goat anti mouse IgG antibody coated plates:***

- ***prevent antibody denaturation as a result of direct adsorption to polystyrene***
- ***unlike Protein A or G plates, these plates bind only to target IgG species***
- ***these plates show a higher antibody-binding capacity than direct adsorption onto polystyrene when using diluted mouse IgG solutions***

### **Product specifications**

#### **Components**

Individually pouched 96-well microplates, configured in 12 removable 8-well strips.

#### **Coating**

Affinity purified goat anti mouse IgG Fc $\gamma$  (subclasses 1+2a+2b+3) is coated using 100  $\mu$ l/well. The strips are post-coated (blocked) for low non specific binding and long-term stability.

#### **Binding capacity**

Microplate was saturated with mouse IgG at a concentration of 1.0  $\mu$ g/ml (100 ng/well) in an ELISA format using goat anti mouse IgG (H+L)-HRP as detector and TMB as substrate (see Figure 1 for data and experiment details).

The Biomat Goat anti mouse IgG Fc $\gamma$  microplate shows a nominal **binding capacity of ~ 0.625 pmol /well of mouse IgG**

#### **Sensitivity**

Mouse IgG was detected at a concentration significantly above background in an ELISA format using goat anti mouse IgG (H+L)-HRP as detector and TMB as substrate (see Figure 1 for data and experiment details).

The Biomat Goat anti mouse IgG Fc $\gamma$  microplate shows a **sensitivity of ~ 0.01  $\mu$ g/ml of mouse IgG**.

#### **Uniformity**

Microplates show a **CV% less than 5** when used as a sandwich of mouse IgG in an ELISA format using goat anti mouse IgG (H+L)-HRP as detector and TMB as substrate.

#### **Storage and Stability**

The microplates, under the indicated storage conditions 2-8 °C, are stable until the expiration date printed on the label.

If opened, store in closed pouch with desiccant and use within the expiration date.

## TECHNICAL NOTE N. 37

### Binding capacity and sensitivity test

1. Add 100  $\mu$ l of different concentrations of mouse IgG (from 0.025 to 4  $\mu$ g/ml) to the wells of goat anti mouse IgG coated plate and incubate for 60 minutes at room temperature
2. Empty the wells and wash with 0.1 M PBS pH 7.2, 0.05% Tween<sup>®</sup> 20 four times
3. Add 100  $\mu$ l /well of Goat anti-mouse IgG (H+ L)-HRP (Jackson ImmunoResearch code 115-035-003, diluted 1: 150.000) and incubate for 30 minutes at room temperature
4. Empty the wells and wash with 0.1 M PBS pH 7.2, 0.05% Tween<sup>®</sup> 20 four times
5. Add 100  $\mu$ l /well of TMB substrate solution and incubate 15 minutes at room temperature
6. Stop the substrate reaction by adding 100  $\mu$ l /well of sulphuric acid 0.3 N and read the optical density values at 450 nm

The data show that a plateau has got starting with an IgG mouse concentration of 1.0  $\mu$ g/ml.

This concentration means the well binding capacity we can express as:

- $\mu$ g/well = 0.1 (100 ng/well)
- pmol/well = 0.625 (this result is calculated considering the IgG M.W. = 160.000)

The microplate sensitivity was calculated as the lowest mouse IgG concentration higher than the mean optical density plus 5 S.D. of 0  $\mu$ g/ml mouse IgG concentration.

Our experiment gave the following results:

- 0  $\mu$ g/ml mouse IgG optical density mean (coming from 8 replicates) = 0.108
- standard deviation = 0.014
- mean + 5 S.D. = 0.178
- sensitivity = 0.012  $\mu$ g/well of mouse IgG

**Figure 1**

