

SECONDARY ANTIBODIES COATED SURFACE: GOAT ANTI RABBIT IgG Fc

The Biomat product is a 96 well coated microplate with goat anti rabbit IgG and a protein to block non-specific binding sites and to maintain stable activity.

Affinity purified goat anti rabbit IgG specifically binds the Fc region of rabbit immunoglobulins, with minimal cross-reaction to human serum proteins.

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 rabbit 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 rabbit solutions

Product specifications

Components

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

Coating

Affinity purified Goat anti rabbit IgG Fc is coated using 100 μ I/well. The strips are post-coated (blocked) for low non specific binding and long-term stability.

Binding capacity

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

The Biomat Goat anti rabbit IgG Fc microplate shows a nominal **binding capacity of ~ 0.625 pmol /well of rabbit IgG**

Sensitivity

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

The Biomat Goat anti rabbit IgG Fc microplate shows a sensitivity of ~ 0.01 µg/ml of rabbit IgG.

Uniformity

Microplates show a $\mathbb{CV\%}$ less than 5 when used as a catcher of rabbit IgG in an ELISA format using goat anti rabbit 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. 38

Binding capacity and sensitivity test

- 1. Add 100 μ l of different concentrations of rabbit IgG (from 0.025 to 4 μ g/ml) to the wells of goat anti rabbit 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 $^{\circledR}$ 20 four times
- 3. Add 100 μ l /well of Goat anti-rabbit IgG (H+ L)-HRP (Jackson ImmunoResearch code 111-035-003, 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[®] 20 four times
- 5. Add 100 µl /well of TMB substrate solution and incubate 15 minutes at room temperature
- 6. Stop the substrate reaction by adding 100 μ l /well of sulphuric acid 1 N and read the optical density values at 450 nm

The data show that a plateau has got starting with an IgG rabbit concentration of 1.0 µg/ml.

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

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

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

Our experiment gave the following results:

- $0 \mu g/ml$ rabbit IgG optical density mean (coming from 8 replicates) = 0.121
- standard deviation = 0.013
- mean + 5 S.D. = 0.186
- sensitivity = $0.010\mu g/well$ of rabbit IgG

Figure 1

Binding capacity and sensitivity of goat anti rabbit IgG coated plate

