JACALIN COATED SURFACE

The Biomat product is a 96 well coated microplate with Jacalin and a surfactant to block non-specific binding sites and to maintain stable activity.
Jacalin, belonging to the lectins family, is a tetrameric (MW 40.000) carbohydrate-binding protein isolated from the seeds of jack fruit Artrocarpus integrifolia agglutinin. It is well known that lectins have been used extensively for the isolation of glycol-conjugates and glycoproteins with specific carbohydrate structures.
Jacalin shows specific affinity for molecules containing non reducing α-D-galactosyl groups, usually present in the biochemical structure of IgA1 and cell membranes.

Example of applications:

- *Human IgA1 specific binding, sterically oriented*
- *Purification of human immunoglobulins (especially IgA1)*
- *Separation of immunocomplexes antigen-antibody*
- *Separation of IgA1 from contaminants*
- *Stimulation of T-cells*

Lots are tested and certified for

- **Uniformity**
- **Binding specificity**
- **Reproducibility**

Product specifications

Components

Individually pouched 96-well microplates, configured in 12 removable 8-well strips or solid format

Coating

Jacalin (mol. Weight 40 kDa) , from the seeds of jack fruit Artrocarpus integrifolia, is coated using 100 µl/well. The strips are post-coated (blocked) for low non specific binding and long-term stability.

Binding capacity

Microplate was saturated with biotinylated human IgA at a concentration of 6 µg/ml (600 ng/well) in an ELISA format using Streptavidin-HRP as detector and TMB as substrate (see Figure 1 for data and experiment details).

The Biomat Jacalin microplate shows a nominal **binding capacity of ~ 4 pmol IgA/well**.

Sensitivity

Biotinylated human IgA was detected at a concentration significantly above background in an ELISA format using streptavidin-HRP as detector and TMB as substrate (see Figure 2 for data and experiment details).
The Biomat Jacalin microplate shows a **sensitivity of 0.0161 µg/ml (1.61 ng/well) of human IgA**.

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Uniformity

Microplates show a **CV% less than 5** when used as a catcher of biotinylated human IgA in an ELISA format using streptavidin-HRP as detector and TMB as substrate.

Storage and Stability

The microplates, if unopened, are stable refrigerated until the expiration date printed on the label. If opened, store in closed pouch with dessicant and use within the expiration date.

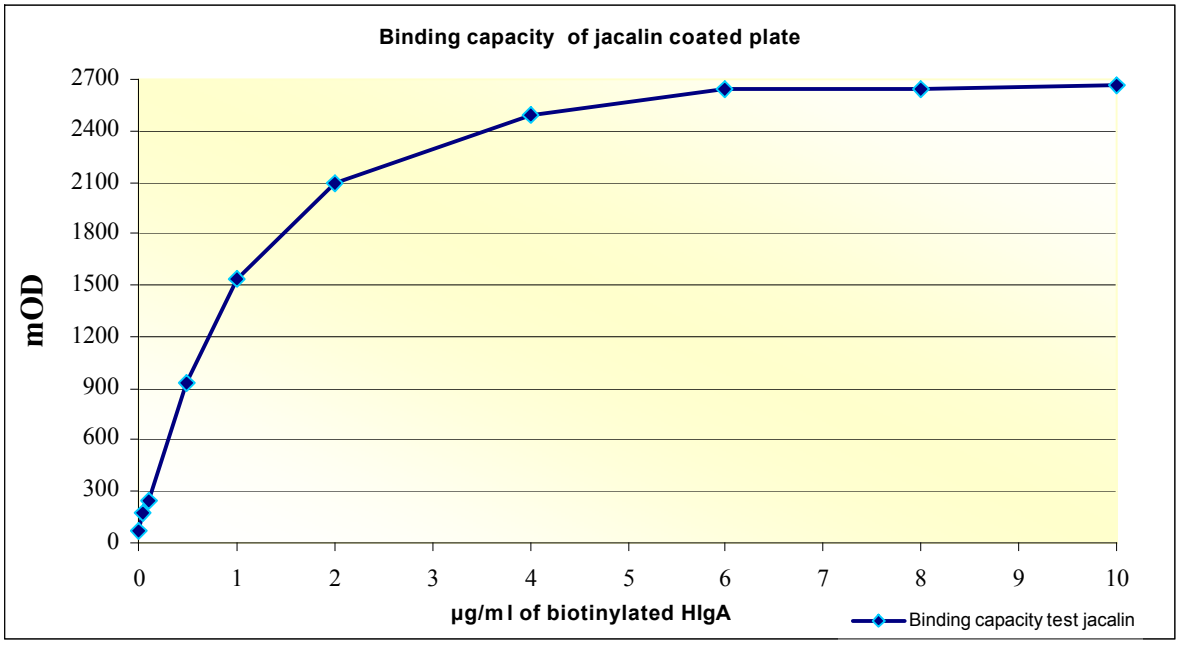
TECHNICAL NOTES N. 28 binding capacity test

Binding capacity test

1. Add 100 µl of different concentrations of biotinylated human IgA (from 0.05 to 10 µg/ml) to the wells of Jacalin coated plate diluted in pure distilled water containing 1 mM $CaCl_2.2\ H_2O$ + 1 mM $MnCl_2.4\ H_2O$. Incubate for 60 minutes at room temperature.
2. Empty the wells and wash with 0.1 M PBS pH 7.2,0.05% Tween 20® four times Add 100 µl /well of Streptavidin-HRP (BioSpa product code SB01-61 , diluted 1:20.000 in pure distilled water containing 1 mM $CaCl_2.2\ H_2O$ + 1 mM $MnCl_2.4\ H_2O$) and incubate for 30 minutes at room temperature.
3. Empty the wells and wash with 0.1 M PBS pH 7.2,0.05% Tween 20® four times
4. Add 100 µl /well of TMB substrate solution and incubate 5 minutes at room temperature.
5. Stop the substrate reaction by adding 100 µl /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 a biotinylated human IgA concentration of 6 µg/ml.
This concentration means the well binding capacity we can express as :
µg/well = 0.6 (600 ng/well)
pmol/well= 4.0 (this result is calculated considering the IgA1 M.W. = 150.000)

Figure 1



TECHNICAL NOTES N. 29 sensitivity test

1. Add 100 µl of different concentrations of biotinylated human IgA (from 0.05 to 10 µg/ml) to the wells of Jacalin coated plate diluted in pure distilled water containing 1 mM $CaCl_2.2\ H_2O$ + 1 mM $MnCl_2.4\ H_2O$ 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 20® four times
3. Add 100 µl /well of Streptavidin-HRP (BioSpa product code SB01-61 , diluted 1:20.000 in pure distilled water containing 1 mM $CaCl_2.2\ H_2O$ + 1 mM $MnCl_2.4\ H_2O$) 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 5 minutes at room temperature.
6. Stop the substrate reaction by adding 100 µl /well of sulphuric acid 0,3 N and read the optical density values at 450 nm

The microplate sensitivity was calculated as the lowest biotinylated IgA concentration higher than the mean optical density plus 5 S.D. of 0 µg/ml biotinylated IgA concentration.
Our experiment gave the following results :

- 0 µg/ml biotinylated IgA optical density mean (coming from 4 replicates) = 0.0655
- standard deviation = 0.0050
- mean + 5 S.D. = 0.0905
- sensitivity = 0,0161 µg/ml (1.61 ng/well) of biotinylated human IgA

Figure 2