MOUSE MONOCLONAL ANTIBODY ISOTYPING COATED SURFACE

The Biomat product is a 96 well coated microplate with goat anti mouse Igs Fc specific (IgG subclasses 1, 2a, 2b, 3 and IgA and IgM classes) and goat anti mouse light chain (kappa and lambda) and a protein to block non-specific binding sites and to maintain stable activity.
Biomat Mouse Monoclonal Antibody Isotyping microplate enables easy identification of mouse immunoglobulin class, subclass and light chain. The microplate can be used performing an ELISA strip-well plates with individual wells precoated with goat anti-mouse Igs Fc specific capture antibody (anti IgG1, IgG2a, IgG2b, IgG3, IgA, IgM) and goat anti-mouse light chain (kappa and lambda) capture antibody. A mouse monoclonal antibody sample applied to the wells can be isotyped within two hours. Results can be evaluated qualitatively by visual inspection or quantitatively by measuring the absorbance at 450 nm (i.e. if using a goat anti-mouse HRP conjugate as detector).

Features of mouse monoclonal antibody isotyping coated plates:

- eight well strip format allows a convenient partial use of the plate; use one strip (column) for each sample (12 samples per plate);
- characterize specific antibodies for six different subclasses and two different light-chain types;
- accurate specificity characterization with samples containing at least 0.05-0.1 µg/ml of IgG1 – $IgG2_a$ – $IgG2_b$ – IgG3 antibody and 0.1 – 0.2 µg/ml of IgM – IgA antibody;
- high compatibility using samples coming from hybridoma cell culture supernatant, ascites fluid or purified antibodies;
- no special equipment is needed to process the microplate; assess results qualitatively evaluated visually or measured quantitatively using an ordinary ELISA plate reader

Product specifications

Available configurations
96-well microplates with 12 removable 8-well strips.

8-well strip coating
Affinity purified goat anti mouse Igs Fc specific (IgG subclasses 1, 2a, 2b, 3 and IgA and IgM classes) and affinity purified goat anti mouse light chain (kappa and lambda) are coated using 100 µl/well, vertically from position A to position H. The strips are post-coated (blocked) for low non-specific binding and long-term stability.

Specificity and reactivity
Mouse Igs were detected at a concentration significantly above background in an ELISA format using goat anti mouse Igs (G/A/M)-HRP as detector and TMB as substrate (see relative figures from 1 to 6 for data and see technical note 43 for experiment details).

Uniformity
All wells show a **CV% less than 10** when used as a sandwich of mouse Igs in an ELISA format using goat anti mouse Igs (G/A/M)-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 NOTES N. 43 – specificity and reactivity test

Six mouse monoclonal antibodies were analyzed to prove the specificity along with the reactivity of the strips.
The six antibodies were respectively:

- IgG 1 kappa (*BioLegend* code 401401)
- IgG 2a kappa (*BioLegend* code 401501)
- IgG 2b kappa (*BioLegend* code 401201)
- IgG 3 kappa (*BioLegend* code 401301)
- IgA kappa (*Invitrogen* code 14-4762-81)
- IgM lambda (*Novus Biologicals* code NBP1-96976)

diluted at 0.1 µg/ml Sample Diluent (*Biomat* code 400-1*)*

Each antibody was then tested according the following method:

1. Add 100 µl of each diluted monoclonal mouse to the wells of one strip (from well A to well H and incubate for 60 minutes at room temperature;
2. Empty the wells and wash with Wash Buffer (*Biomat* code 200-3*)* four times;
3. Add 100 µl/well of goat anti-mouse Igs (G/A/M)-HRP (*Southern Biologicals* code 1010-05) diluted 1:10,000 in Diluent for HRP conjugate (*Biomat* code 400-2) and incubate for 30 minutes at room temperature;
4. Empty the wells and wash with Wash Buffer (*Biomat* code 200-3*)* four times;
5. Add 100 µl/well of TMB substrate solution (*Biomat* code 500-1) and incubate 15 minutes at room temperature;
6. Stop the substrate reaction by adding 100 µl/well of sulphuric acid, (*Biomat* code 600-1) and read the optical density values at 450 nm

The graphs that express the reactivity of each control and its specificity are shown in the following figures.

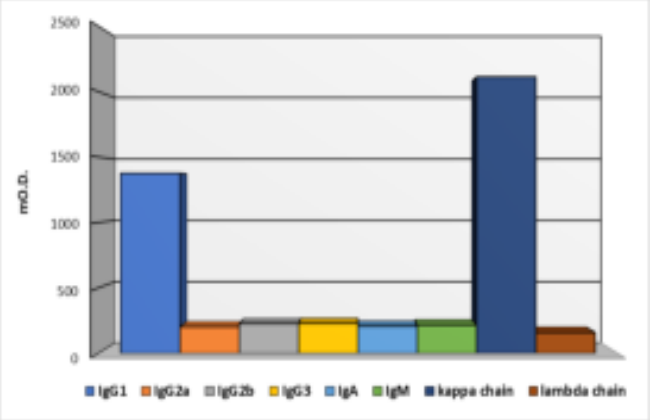

Figure 1: the graph shows the results, expressed in mO.D., of the analysis of the monoclonal control IgG1 kappa (*BioLegend* code 401401)

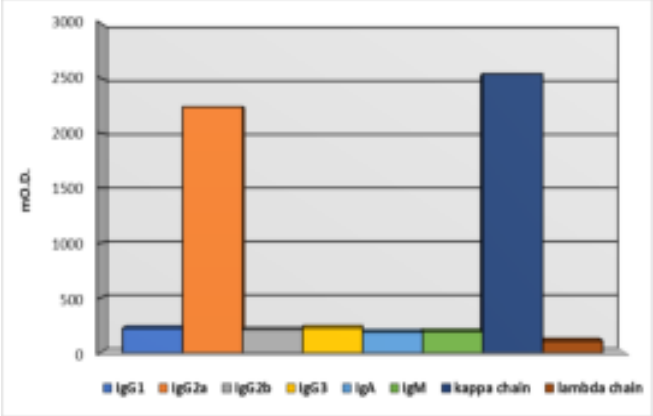

Figure 2: the graph shows the results, expressed in mO.D., of the analysis of the monoclonal control IgG2a kappa (*BioLegend* code 401501)

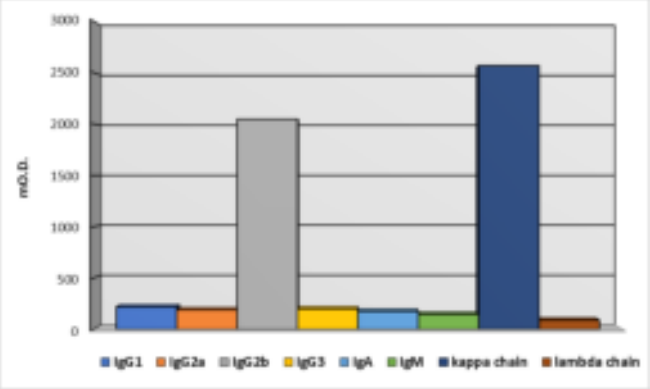

Figure 3: the graph shows the results, expressed in mO.D., of the analysis of the monoclonal control IgG2b kappa (*BioLegend* code 401201)

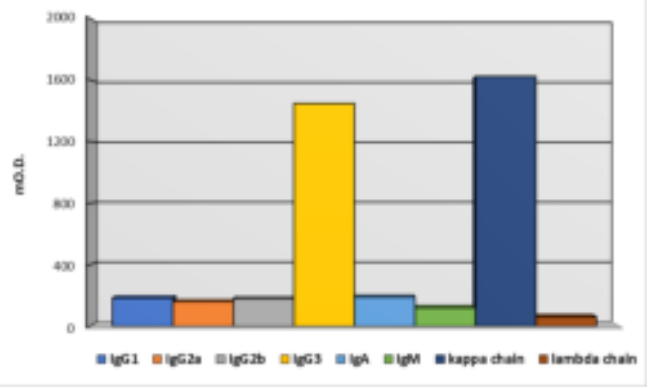

Figure 4: the graph shows the results, expressed in mO.D., of the analysis of the monoclonal control IgG3 kappa (*BioLegend* code 401301)

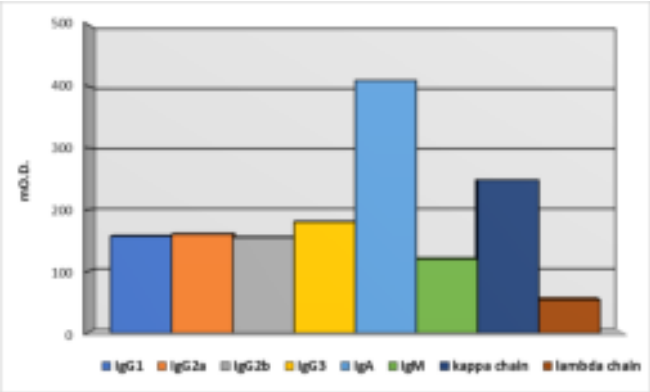

Figure 5: the graph shows the results, expressed in mO.D., of the analysis of the monoclonal control IgA kappa (*Invitrogen* code 14-4762-81)

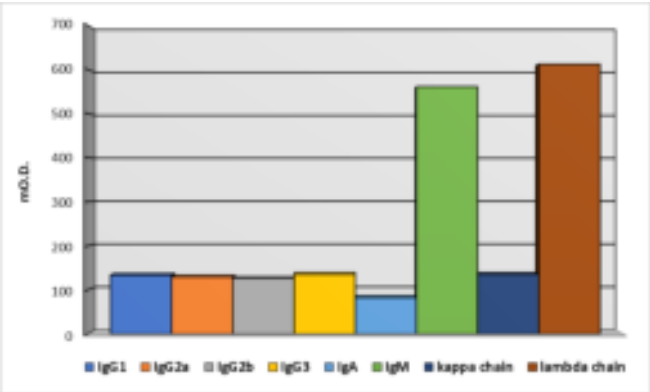

Figure 6: the graph shows the results, expressed in mO.D., of the analysis of the monoclonal control IgM lambda (*Novus Biologicals* code NBP1-96976)