

MOUSE MONOCLONAL ANTIBODY ISOTYPING COATED SURFACE

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.

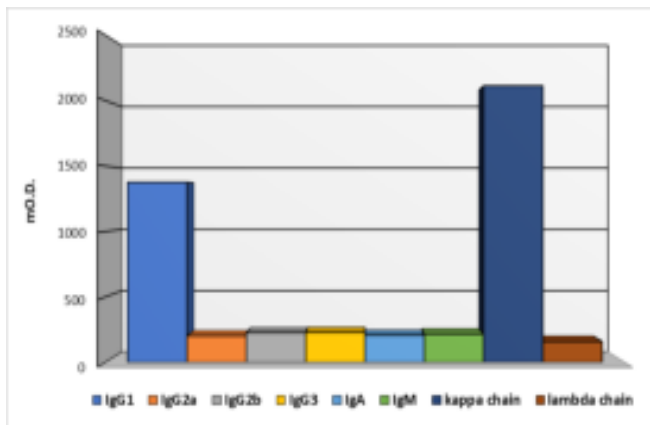


Figure 1: the graph shows the results, expressed in m.O.D., of the analysis of the monoclonal control IgG1 kappa (*BioLegend* code 401401)

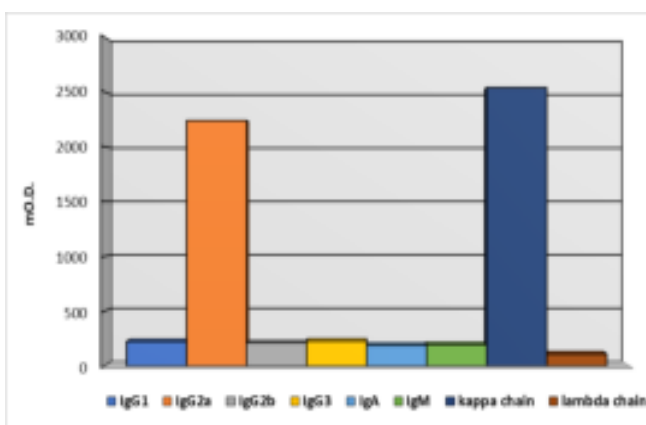


Figure 2: the graph shows the results, expressed in m.O.D., of the analysis of the monoclonal control IgG2a kappa (*BioLegend* code 401501)

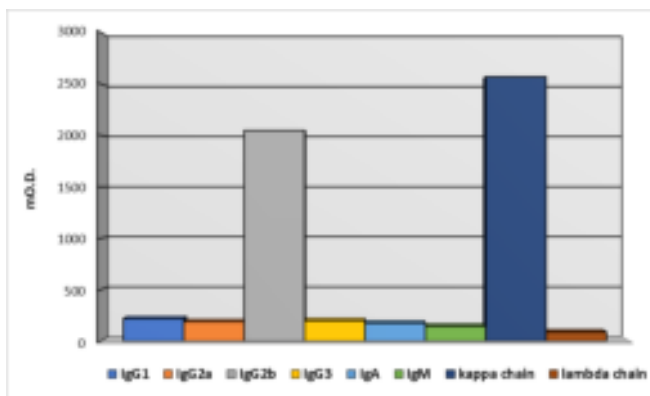


Figure 3: the graph shows the results, expressed in m.O.D., of the analysis of the monoclonal control IgG2b kappa (*BioLegend* code 401201)

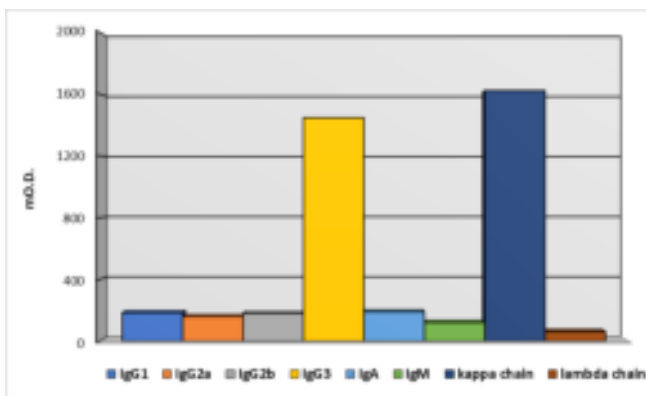


Figure 4: the graph shows the results, expressed in m.O.D., of the analysis of the monoclonal control IgG3 kappa (*BioLegend* code 401301)

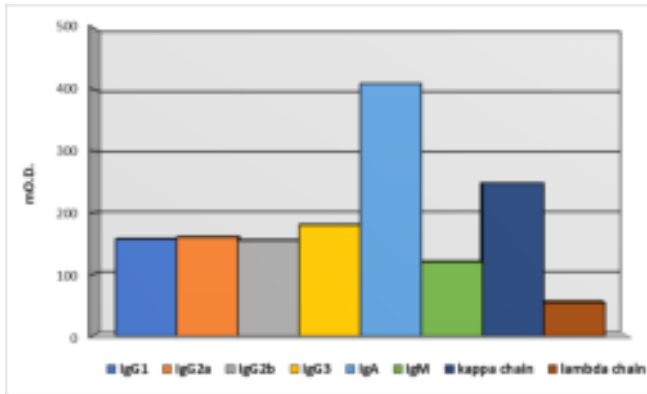


Figure 5: the graph shows the results, expressed in mO.D., of the analysis of the monoclonal control IgA kappa (Invitrogen code 14-4762-81)

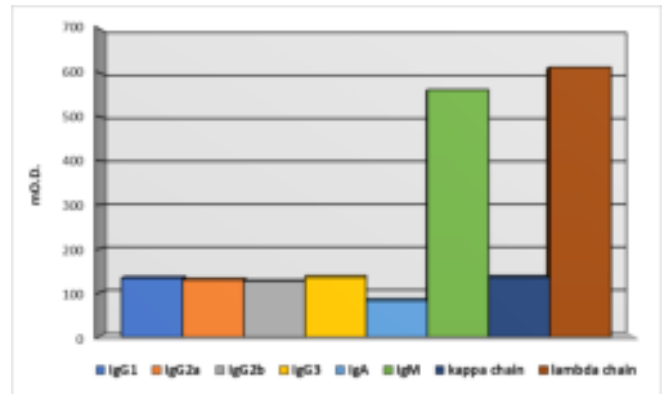


Figure 6: the graph shows the results, expressed in mO.D., of the analysis of the monoclonal control IgM lambda (Novus Biologicals code NBP1-96976)