

Mouse Monoclonal Antibody Isotyping ELISA

Reagents:

- Goat anti-mouse subclass specific antibodies (Sigma, ISO2)
- Peroxidase-conjugated goat anti-mouse IgG(H+L) (Jackson ImmunoResearch, 115-035-062)

Buffers:

- Carbonate buffer (15 mmol Na₂CO₃, 35 mmol NaHCO₃, pH 9.6)
- Phosphate buffer (2.5 mmol NaH₂PO₄, 7.5 mmol Na₂HPO₄, 145 mmol NaCl, 0.05% (v/v) Tween 20, pH 7.2)
- Substrate buffer (33.3 mmol citric acid, 66.7 mmol NaH₂PO₄, pH 5.0)
- Stop solution (1N H₂SO₄)
- Substrate solution (make fresh before use): Substrate buffer supplemented with 130ug/ml of 3,3',5,5'-tetramethylbenzidine (TMB, Sigma T5525-100TAB) and 0.01% (v/v) hydrogen peroxide.

Procedure:

1. Coating of the plates:
 - a. Use goat anti-Mouse IgG1, IgG2a, IgG2b, IgG3, IgA, or IgM (1:1000) in carbonate buffer for coating
 - b. Coat with 100µl per well
 - c. Incubate the ELISA plate overnight at 4°C or 1h at 37°C
2. Wash the coated plate three times with 300µl/well phosphate buffer.
3. Add 50µl of undiluted sample (cell culture supernatants) to each well. Add phosphate buffer instead of the sample to 1-2 wells per isotype to serve as background control. (Cell culture supernatants can also be diluted for quantification in this step. A mouse monoclonal antibody of the corresponding isotype and known concentration can be used as standard for quantification of the unknown mab concentration. Use a titration of both mabs for the quantification.)
4. Incubate for 90 minutes at room temperature.
5. Wash the coated plate three times with 300µl/well phosphate buffer.
6. Add 50µl/well of peroxidase-conjugated goat anti-mouse IgG(H+L) diluted 1:20,000 (or 25µg/ml) in phosphate buffer.
7. Incubate for 30 minutes at room temperature.
8. Wash plate three times with 300µl/well phosphate buffer.
9. Add 50µl fresh substrate solution per well
10. Incubate for 10-20 minutes in the dark at room temperature.
11. Stop the colorimetric reaction by adding 50µl 1N H₂SO₄ per well
12. Read plate at 450nm absorbance in an automated microplate reader.