

DNP COATED SURFACE

TECHNICAL NOTE N. 40

Binding capacity test

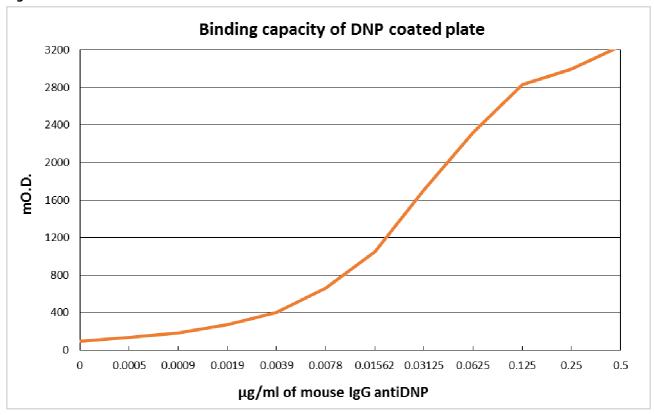
- Add 100 µl of different concentrations of monoclonal mouse IgG anti DNP (Merck-Sigma cat. # Mab 2223 at 1 mg/ml), from 0.0005 to 0.500 µg/ml, diluted in Sample Diluent, (Biomat code 400-1-100) to the wells of DNP coated plate and incubate for 60 minutes at room temperature
- 2. Empty the wells and wash with Wash Buffer, (Biomat code 200-1-100) four times
- 3. Add 100 µl /well of goat anti-mouse IgG -HRP (*Jackson ImmunoResearch* code 115-035-003), diluted 1:25,000 in Diluent for HRP conjugate, (*Biomat* code 400-2-100) and incubate for 60 minutes at room temperature
- 4. Empty the wells and wash with Wash Buffer, (Biomat code 200-1-100) four times
- 5. Add $100 \ \mu l$ /well of TMB substrate solution, (*Biomat* code 500-1-100) and incubate 15 minutes at room temperature
- 6. Stop the substrate reaction by adding 100 μ l /well of sulphuric acid, (*Biomat* code 600-1-100) and read the optical density values at 450 nm

The data show that a plateau has got starting with a mouse IgG anti DNP concentration of 0.125 µg/ml.

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

 $\mu g/well = 0.0125 (12.5 \text{ ng/well})$

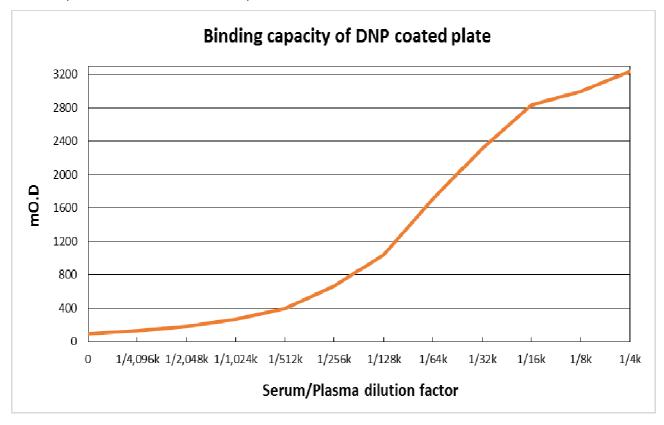
Figure 1



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Figure 2: the figure gives an idea of the dilution factor to apply to the serum/plasma of the immunized mouse under evaluation; where k means a dilution of 1:1,000



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