TNP COATED SURFACE

TECHNICAL NOTE N. 41

Binding capacity test

1. Add 100 µl of different concentrations of monoclonal mouse IgG anti TNP, from 0.0005 to 0.500 µg/ml, diluted in Sample Diluent, (*Biomat* code 400-1-100) to the wells of TNP 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 µl/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 µl/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 TNP concentration of 0.250 µg/ml.

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

- µg/well = 0.0250 (25 ng/well)

Figure 1

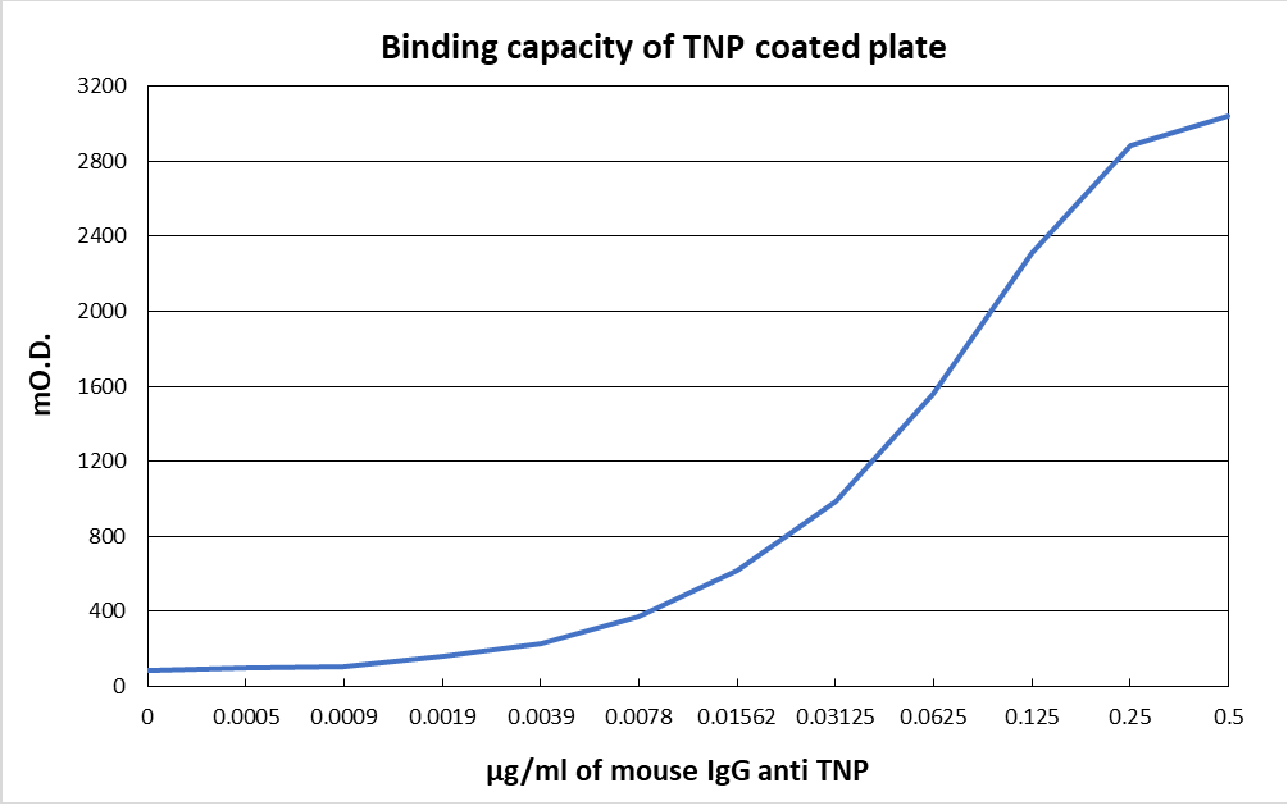

Figure 2: the figure gives an idea of the dilution factor to apply to the serum/plasma of the immunized mouse under evaluation; where k means a dilution of 1:1,000