

## TECHNICAL NOTE N. 40

### Binding capacity test

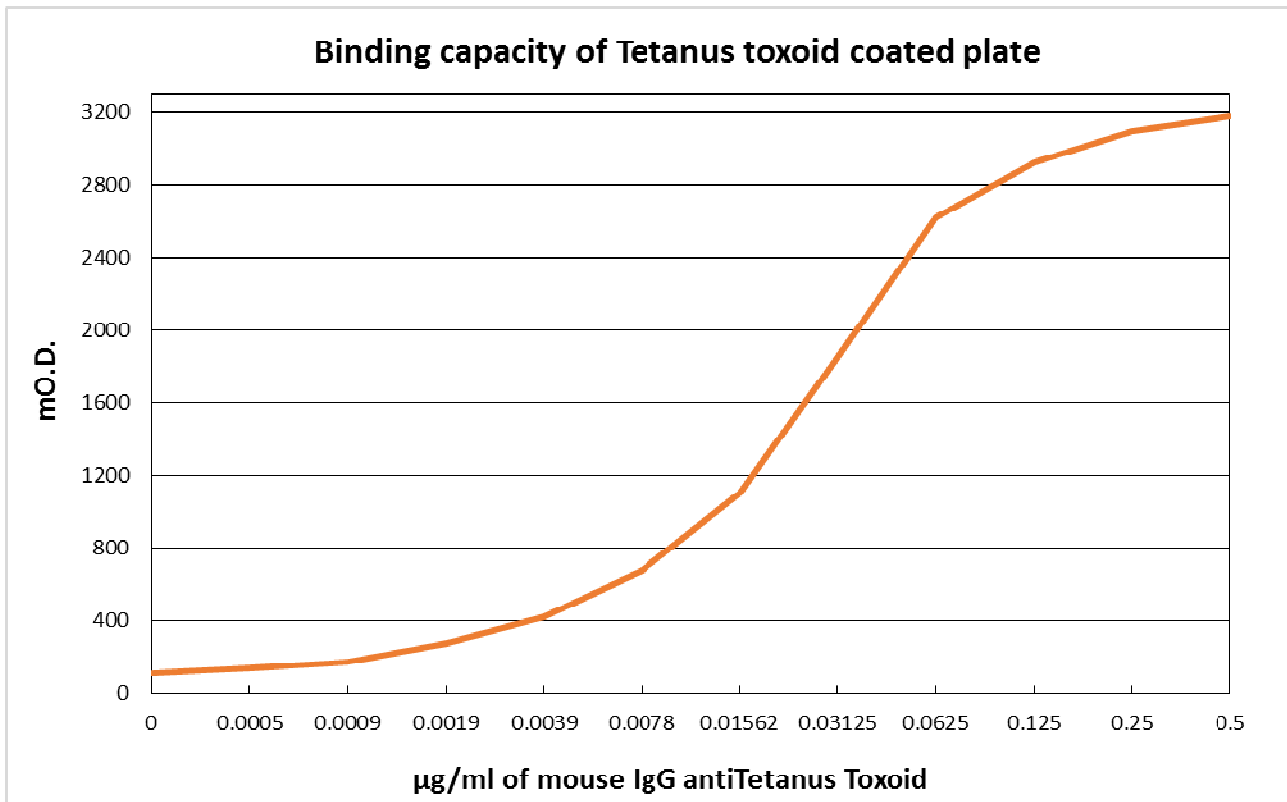
1. Add 100  $\mu$ l of different concentrations of monoclonal mouse IgG anti Tetanus Toxin (*NovusBio* code NBP1-05447 at 10.7 mg/ml), from 0.0005 to 0.500  $\mu$ g/ml, diluted in Sample Diluent, (*Biomat* code 400-1-100) to the wells of Tetanus Toxoid coated plate and incubate for 60 minutes at room temperature
2. Empty the wells and wash with Wash Buffer, (*Biomat* code 200-1-100) four times
3. Add 100  $\mu$ l/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  $\mu$ 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 Tetanus Toxoid concentration of 0.125  $\mu$ g/ml.

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

- $\mu$ g/well = 0.0125 (12.5 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

