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NB100-64513 Protocol

ELISA protocol (NB100-64513)

1. Coating Buffer
Na2CO3, 1.5 g
NaHCO3, 2.93 g
Distilled water, 1 liter, pH to 9.6

2. Blocking buffer
Phosphate Buffered Saline (PBS) containing 1% w/v BSA

3. Wash buffer
Phosphate Buffered Saline containing 0.05% v/v Tween-20

Method

1. Coat microtiter plate wells with 100 ul of the appropriate coating antigen, at a concentration of between 1-10 ug/ml in coating buffer. Cover the plate and incubate overnight at 4C. Wash the plate 3 times in wash buffer.

2. Add 150 ul of blocking solution to each well. Incubate for 60 minutes at 37C. Wash 4 times in wash buffer.

3. Add 100 ul of suitably diluted samples to the relevant wells. Ensure that appropriately diluted standards are included (dilute samples and standards in wash buffer). Samples or standards should preferably be run in triplicate. Incubate for 90 minutes at 37C or overnight at 4C. Wash 3 times in wash buffer.

4. Add 100 ul of biotin-conjugated detection antibody (appropriately diluted in wash buffer) to each well. Incubate for 1 hour at 37C. Wash 3 times in wash buffer.

5. Add 100 ul of enzyme-conjugated streptavidin (appropriately diluted in wash buffer) to each well. Incubate for 60 minutes at 37C. Wash 3 times in wash buffer.

6. Add 100 ul of the appropriate substrate solution1 to each well. Incubate at room temperature (and in the dark if required) for 30 minutes, or until desired color change is attained.

7. Read absorbance values immediately at the appropriate wavelength.

8. OR add 50 ul of stop solution. Gently tap plate to ensure thorough mixing. Measure absorbance within 30 minutes.