
ELISA-LIKE ASSAY

MATERIALS:

- 96well-plate Maxisorp from Nunc
- ELISA Diluent Solution (5X) (eBioscience 00-4202), diluted 1/5 in water
- Avidin HRP (eBioscience 18-4100),
- 1X TMB ELISA Substrate Solution (eBioscience 00-4201)
- Bovine serum albumin diluted at 2%-3% in water
- Phosphate buffer saline solution (10X) (used at stock concentration)
- Gelatin nanoparticles (at 0.1 g/ml)
- Biotinylated gelatin nanoparticles (at 0.1 g/ml)

PROCEDURE:

- Coat a 96 wells UV plate as follows:

A	B	C	D	E	F	G	H
45 μ L NPs + 5 μ L PBS	45 μ L biot. NPs + 5 μ L PBS	50 μ L PBS	nothing	45 μ L BSA + 5 μ L PBS	50 μ L BSA	50 μ L NPs	50 μ L biot. NPs

- Cover with parafilm and incubate overnight at +4°C.
- Discard the content of the wells.
- Wash 3 times with ELISA wash buffer.
- Add 100 μ L/well of blocking solution.
- Incubate for 1-2 hours at room temperature or overnight at +4°C.
- Wash again 3 times.
- Add 50 μ L/well of avidin-HRP and incubate for 30 min at room temperature.
- Wash again 5 times.
- Add 50 μ L/well of TMB and incubate for 15-30 min at room temperature.
- Under a hood, add 50 μ L/well of 2N H₂SO₄ (ELISA blocking solution).
- Read the plate at 412 nm.