



Title: *ELISA to detect anti-Substance P antibody*

No: RTLP-GL-EL2

Location:
Old CCRC Tripp Lab

Approval Date:
10 September 2004

Supersedes Date:

Materials:

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|-----------------|--|----------------------------------|------------------|
| •Lab coat | •Dulbecco's Phosphate-Buffered Saline (DPBS) | •Streptavidin-coated microplates | •Pipettes |
| •Gloves | | •Biotinylated- | •Pipetteman |
| •Tween 20 | | substance P (SP-B) | •Pipette Aid |
| •Gelatin | •Horse-radish peroxidase (HRP)-labeled anti-rat Ab | •Rat anti-substance P | •Pipetteman tips |
| •Paper towel | | •H ₃ PO ₄ | •TMB Substrate |
| •37°C incubator | | | |

Procedure:

1. Prepare buffers:
 - ELISA buffer: DPBS + 0.05% tween-20 (DPBSt)
 - Blocking buffer: DPBSt + 0.5% gelatin
2. Dilute STOCK biotinylated Substance P (SP-B; currently at 9.6 mg?) in ELISA buffer to a concentration of 1mg/ml.
3. Make a series of 10-fold dilutions from the 1mg/ml SP-B in ELISA buffer: 100, 10, 1, 0.1, 0.01, 0.001, 0.0001 µg/ml.
4. Add 100 µl of diluted SP-B to wells of two streptavidin-coated plates and incubate one of the plates for 1 hour at 37 °C and the second plate for 1 hour at 4°C.
5. Following incubations, remove supernatants by flicking plates onto paper towel. Wash vigorously with ELISA blocking buffer 5 to 6 times.
6. Add 100 µl of the positive control antibody (purified rat anti-SP antibody).
7. Make dilutions of serum to be analyzed for anti-SP antibodies in ELISA buffer (1:10, 1:50, 1:100, 1: 200, 1:500); add 100 µl/well
8. Incubate for 1 hour at 37 °C or 4°C (you will be testing two plates as in step 4).

9. Repeat step 5.
10. Dilute HRP-labeled anti-rat antibody in ELISA buffer to 1 µg/mL and add 100 µL of diluted HRP-labeled anti-rat to wells.
11. Incubate both plates for 1 hour at 37 °C.
12. Repeat step 5.
13. Add 150 µl of TMB substrate to wells. Read after 10-15 minutes or stop the reaction by adding 70 µl of dilute H₃PO₄.
14. Read optical density (OD) at 450 nm.

Author	Management Approval/Date	Effective Date