



Title: *Immunohistochemistry (IHC) for LabTek Slides*

No: RTLP-IH-2

Location:
Old CCRC Tripp Lab

Approval Date:
10 September 2004

Supersedes Date:

Materials:

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|-----------------------------------|---------------------------|--------------------|-------------------|
| •Lab coat | •Phosphate Buffered | •Antibodies: | •Pipettes |
| •Gloves | Saline (PBS) | 1°-aMPV peptide | •Pipetteman |
| •3% H ₂ O ₂ | •Tween-20 | 2°-biotinylated | •Pipette Aid |
| • Sink with running | •37°C, 5% CO ₂ | α-rabbit IgG | •Pipetteman tips |
| H ₂ O | incubator | •dH ₂ O | •Centrifuge |
| •Powerblock blocking | •3, 3' diamino- | •Vectastain ELITE | •4°C cold storage |
| solution | benzidine (DAB) | ABC kit | •Hematoxalin |

Procedure:

1. Quench endogenous peroxidase with 3% H₂O₂ for 10 min at room temperature. Wash in running water for 10 minutes.
2. Block for 5 minutes at 37°C with Powerblock blocking solution
3. Drain slides. Add 1° antibody (aMPV/N peptide Ab) diluted 1:500 in PBS-0.5% Tween. Incubate for 2 hours at 37°C or overnight at 4°C.
4. Add PBS-0.5% Tween 2 X 5 min.
5. Add 2° antibody-biotinylated anti-Rabbit IgG diluted 1:250 in PBS-0.5% Tween, incubate for 1 hour at 37°C.
6. Add PBS-0.5% Tween 2 X 5 minutes.
7. Incubate 1 hour at 37°C with Elite peroxidase: Add 2 drops A to 5 ml PBS-0.5% Tween, mix. Add 2 drops of B. (Make at least 30 min before use).
8. Add PBS-0.5% Tween 2 X 5 min.

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9. Add substrate, DAB. 3, 3' diaminobenzidine . To 5 ml H₂O, add 2 drops buffer, 4 drops of DAB, and 2 drops of H₂O₂.
10. Develops in 2-10 minutes, watch closely!
11. Counterstain with Hematoxylin and coverslip.