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NB300-232 Protocol

Protocol specific for SCP3 Antibody (NB300-232)

SCP3/SYCP3 Antibody: https://www.novusbio.com/products/scp3-sycp3-antibody_nb300-232
Immunofluorescence Procedure

1. Freshly prepared slides are soaked in 1X ADB for 75 minutes.
2. Primary antibodies are added concurrently (SCP3 and CDK2).
3. The primary antibodies are incubated overnight in a hudid chamber (37 degrees Celcius).
4. The slides are washed for 40 minutes in 1X ADB.
5. The slides are detected with the appropriate secondary antibodies (RDAR for SCP1 and FDAM for CDK2).
6. The slides are incubated for 4 hours in a humid chamber (37 degrees Celcius).
7. The slides are washed for 20 minutes in 1X ADB, followed by 3 washes, 10 minutes each, in 1X PBS.
8. The slides are counterstained with DAPI.
9. Images are captured after allowing the slides to remain in the dark overnight at RT.

Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.