Product Datasheet

NIK/MAP3K14 Antibody - BSA Free NBP2-23603-0.1ml

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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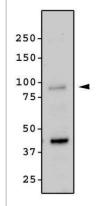


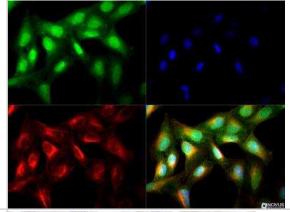
Images

Western Blot: NIK/MAP3K14 Antibody [NBP2-23603] - Detection of MAP3K14 in U2OS cell lysate.

Immunocytochemistry/Immunofluorescence: NIK/MAP3K14 Antibody [NBP2-23603] - MAP3K14 antibody was tested in U2OS cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with

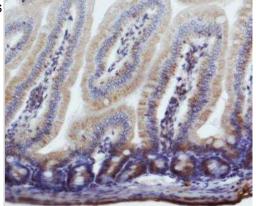
DAPI (blue) and Dylight 550 (red).





Immunohistochemistry: NIK/MAP3K14 Antibody [NBP2-23603] - Analysis of MAP3K14 in mouse small intestine.

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Publications

Shiode Y, Kodama T, Shigeno S et al. TNF receptor-related factor 3 inactivation promotes the development of intrahepatic cholangiocarcinoma through NF-kappa B-inducing kinase-mediated hepatocyte transdifferentiation Hepatology (Baltimore, Md.) 2022-01-07 [PMID: 34995376] (IHC-P)



Procedures

Western blot Protocol for MAP3K14 Antibody (NBP2-23603)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.

2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.

3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.

4. Rinse the blot.

5. Block the membrane using standard blocking buffer for at least 1 hour.

6. Wash the membrane in wash buffer three times for 10 minutes each.

7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.

8. Wash the membrane in wash buffer three times for 10 minutes each.

9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.

10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).

11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

ICC/IF Protocol for MAP3K14 Antibody (NBP2-23603)

Immunocytochemistry Protocol

Culture cells to appropriate density on suitable glass coverslips in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 5-10 minutes.

2. Remove the formalin and add 0.5% Triton-X 100 in TBS to permeabilize the cells. Incubate for 5-10 minutes.

3. Remove the permeabilization buffer and add wash buffer (i.e. PBS or PBS with 0.1% Tween-20). Be sure to not let the specimen dry out. Gently wash three times for 10 minutes.

4. Alternatively, cells can be fixed with -20C methanol for 10 min at room temperature. Remove the methanol and rehydrate in PBS for 10 min before proceeding.

5. To block nonspecific antibody binding incubate in 10% normal goat serum for 1 hour at room temperature.

6. Add primary antibody at appropriate dilution and incubate at room temperature for 1 hour or at 4 degrees C overnight.

7. Remove primary antibody and replace with wash buffer. Gently wash three times for 10 minutes.

8. Add secondary antibody at the appropriate dilution. Incubate for 1 hour at room temperature.

9. Remove antibody and replace with wash buffer. Gently wash three times for 10 minutes.

10. Nuclei can be staining with 4',6' diamino phenylindole (DAPI) at 0.1 ug/ml, or coverslips can be directly mounted in media containing DAPI.

11. Cells can now be viewed with a fluorescence microscope.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow proper laboratory procedures for the disposal of formalin.



IHC Protocol for MAP3K14 Antibody (NBP2-23603)

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 degrees 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.





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Products Related to NBP2-23603-0.1ml

NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NBP2-23603UV	NIK/MAP3K14 Antibody [DyLight 350]

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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