

Product Datasheet

Cytokeratin 5 Antibody - BSA Free NBP2-61931

Unit Size: 0.1 mg

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

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Publications: 2

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NBP2-61931**Cytokeratin 5 Antibody - BSA Free**

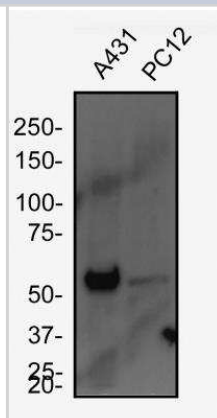
Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Description	Novus Biologicals Rabbit Cytokeratin 5 Antibody - BSA Free (NBP2-61931) is a polyclonal antibody validated for use in IHC, WB, Flow and ICC/IF. Anti-Cytokeratin 5 Antibody: Cited in 2 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	3852
Gene Symbol	KRT5
Species	Human, Rat
Immunogen	Partial recombinant human Cytokeratin 5 protein (amino acids 19-78) [Uniprot: P13647]

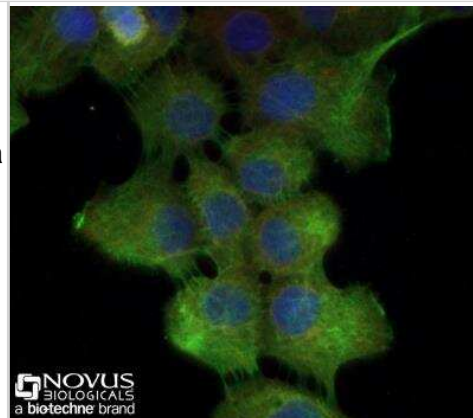
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry
Recommended Dilutions	Western Blot 1 ug/ml, Flow Cytometry 2-10 ug/million cells, Immunohistochemistry 1:1000, Immunocytochemistry/ Immunofluorescence 5 ug/ml, Immunohistochemistry-Paraffin 1:1000

Images

Western Blot: Cytokeratin 5 Antibody [NBP2-61931] - Total protein from human A431 cells and rat PC12 cells was separated on a 7.5% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/ml anti-Cytokeratin 5 in 5% non-fat milk in TBST and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.



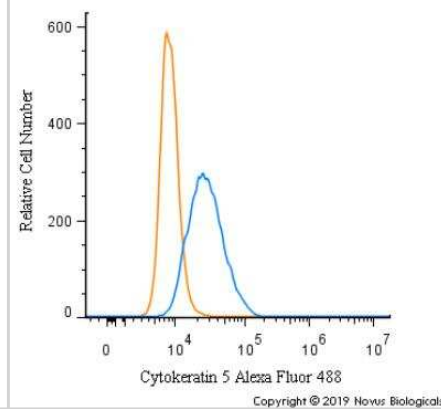
Immunocytochemistry/Immunofluorescence: Cytokeratin 5 Antibody [NBP2-61931] - A431 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.1% Triton X-100. The cells were incubated with anti-Cytokeratin 5 at 5 ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse DyLight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Immunohistochemistry-Paraffin: Cytokeratin 5 Antibody [NBP2-61931] - Analysis of a FFPE tissue section of human skin using 1:1000 dilution of rabbit anti-Cytokeratin 5 antibody. The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin. This Cytokeratin 5 antibody generated a specific membrane cytoplasmic staining in the epithelial cells of the skin tissue. The signal was highest in the keratinocytes while dermis was largely negative for this Cytokeratin 5 (KRT5).



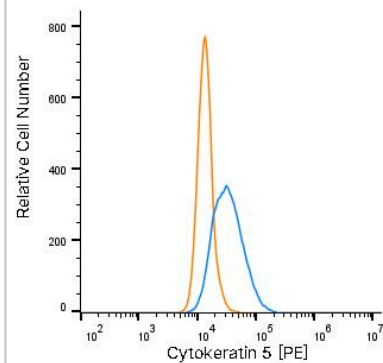
Flow Cytometry: Cytokeratin 5 Antibody [NBP2-61931] - An intracellular stain was performed on A431 cells with Cytokeratin 5 Antibody NBP2-61931AF488 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 10 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.



Cytokeratin 5 was detected in immersion fixed A431 human skin carcinoma cell line using Rabbit anti-Cytokeratin 5 Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NBP2-61931AF647) (red) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.

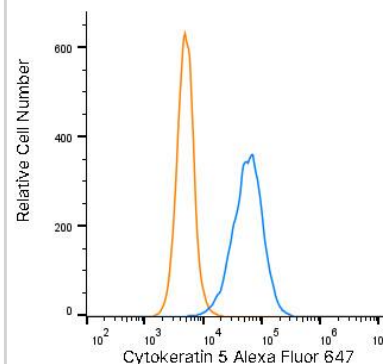


A431 human skin carcinoma cell line was stained with Rabbit anti-Cytokeratin 5 Affinity-purified Polyclonal Antibody conjugated to Phycoerythrin (Catalog # NBP2-61931PE, blue histogram) or matched control antibody (Catalog # NBP2-24983, orange histogram).



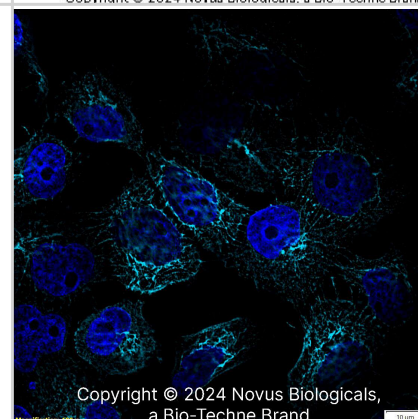
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An intracellular stain was performed on A431 human skin carcinoma cell line using Rabbit anti-Cytokeratin 5 Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NBP2-61931AF647, blue histogram) or matched control antibody (Catalog # NBP2-24981AF647, orange histogram) at 2.5 µg/mL for 30 minutes at RT.



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Cytokeratin 5 was detected in immersion fixed A431 human skin carcinoma cell line using Rabbit anti-Cytokeratin 5 Antigen Affinity Purified Polyclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NBP2-61931AF647) (light blue) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.



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Publications

Redman RS, Alvarez-Martinez JC Identifying stem cells in the main excretory ducts of rat major salivary glands: adventures with commercial antibodies Biotechnic & histochemistry : official publication of the Biological Stain Commission 2023-05-01 [PMID: 36779267]

Castaneda DC, Jangra S, Yurieva M et al. Spatiotemporally organized immunomodulatory response to SARS-CoV-2 virus in primary human broncho-alveolar epithelia bioRxiv : the preprint server for biology 2023-03-31 [PMID: 37034597] (IHC-Fr, Human)

Procedures

Western Blot Protocol for Cytokeratin 5 Antibody (NBP2-61931)

Cytokeratin 5 Antibody:
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute anti-Cytokeratin 5 primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST 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.

Immunohistochemistry-Paraffin Protocol for Cytokeratin 5 Antibody (NBP2-61931)

Cytokeratin 5 Antibody:
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.



Immunocytochemistry/Immunofluorescence Protocol for Cytokeratin 5 Antibody (NBP2-61931)**Cytokeratin 5 Antibody:
Immunocytochemistry Protocol**

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

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeabilization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPI if required.

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





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Products Related to NBP2-61931

NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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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