Product Datasheet

BTK Antibody
NBP1-78295SS

Unit Size: 0.025 ml

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

Publications: 2

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
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Updated 6/15/2014 v.20.1

NBP1-78295SS

BTK [p Tyr223] Antibody

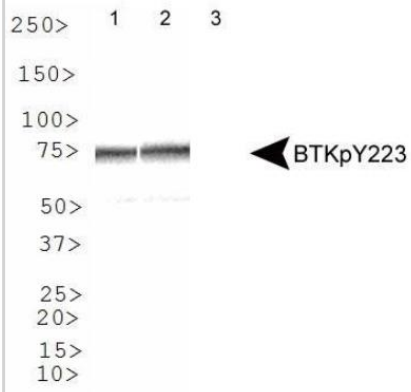
Product Information	
Unit Size	0.025 ml
Concentration	1.09 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Purity	Affinity purified
Buffer	PBS, 30% glycerol

Product Description	
Host	Rabbit
Gene ID	695
Gene Symbol	BTK
Species	Human, Mouse
Species Reactivity	Human and mouse.
Immunogen	A synthetic phosphorylated peptide made to a peptide surrounding amino acid 223 of the human BTK protein [UniProt Q06187]

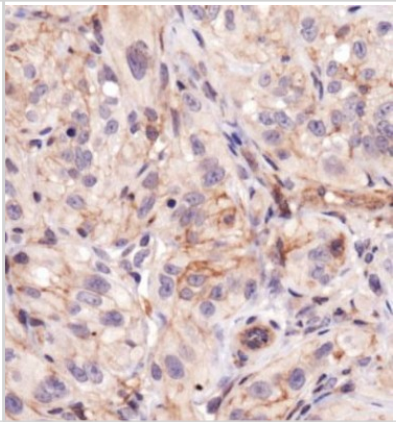
Product Application Details	
Applications	Western Blot, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Immunocytochemistry/Immunofluorescence 1:200, Immunohistochemistry 1:200, Immunohistochemistry-Paraffin 1:200, Western Blot 1 ug/ml
Application Notes	This BTK [pY223] antibody is useful for Western Blot, Immunocytochemistry/Immunofluorescence, and IHC-paraffin embedded sections. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.

Images

Western Blot: BTK [p Tyr223] Antibody [NBP1-78295] - WB analysis of BTKpY223 on Ramos whole cell lysate with peptide competition: 1. ab only, 2. 200 molar excess unmodified immunizing peptide and 3. 200 molar excess modified immunizing peptide.



Immunohistochemistry: BTK [p Tyr223] Antibody [NBP1-78295] - IHC analysis of BTK in kidney cancer xenograft using DAB with hematoxylin counterstain.



Publications

Eda H, Santo L, Cirstea DD et al. A novel Bruton's tyrosine kinase inhibitor CC-292 in combination with the proteasome inhibitor carfilzomib impacts the bone microenvironment in a multiple myeloma model with resultant antimyeloma activity. Leukemia 2/12/2014 [PMID: 24518207] (WB, Human)

Gleixner KV, Peter B, Blatt K, Suppan V et al. Synergistic growth-inhibitory effects of ponatinib and midostaurin (PKC412) on neoplastic mast cells carrying KIT D816V Haematologica 2013 Mar 28 [PMID: 23539538] (WB, Human)

Procedures

Western Blot protocol specific for BTK antibody (NBP1-78295) WB
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%.

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

Immunohistochemistry-Paraffin Embedded Sections protocol specific for BTK antibody (NBP1-78295) IHC-P
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 4C.
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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Immunocytochemistry/Immunofluorescence Protocol for BTK Antibody (NBP1-78295)
Immunocytochemistry Protocol

Culture cells to appropriate density 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 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

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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.

For more information on our guarantee, please visit www.novusbio.com/guarantee.