

# Product Datasheet

## BTK [p Tyr223] Antibody - BSA Free NBP1-78295

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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[technical@novusbio.com](mailto:technical@novusbio.com)

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**NBP1-78295**

BTK [p Tyr223] Antibody - BSA Free

Product Information	
Unit Size	0.1 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
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS and 30% Glycerol

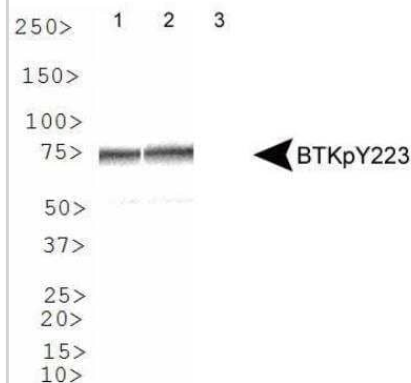
Product Description	
Description	Novus Biologicals Rabbit BTK [p Tyr223] Antibody - BSA Free (NBP1-78295) is a polyclonal antibody validated for use in IHC, WB, Flow and ICC/IF. Anti-BTK Antibody: Cited in 5 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	695
Gene Symbol	BTK
Species	Human, 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, Immunohistochemistry-Paraffin, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry
Recommended Dilutions	Western Blot 1 ug/ml, Flow Cytometry reported in scientific literature (PMID 25724205), Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:200, Immunohistochemistry-Paraffin 1:200
Application Notes	Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.

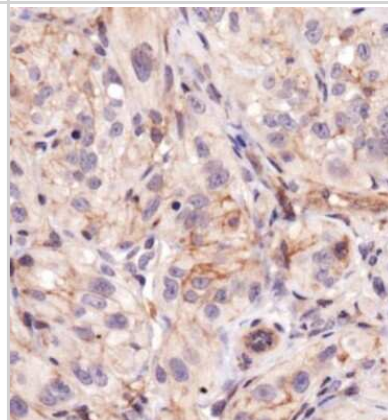


## Images

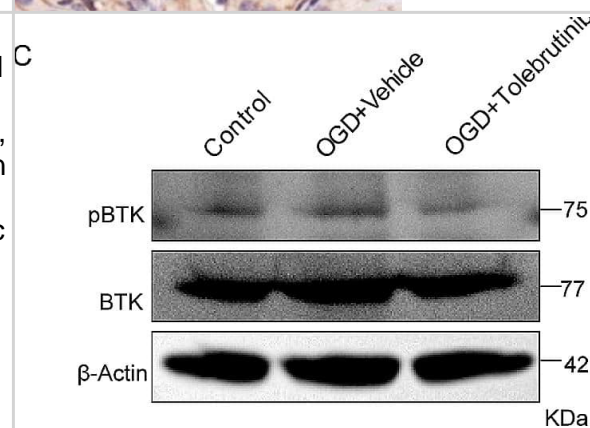
Western Blot: BTK [p Tyr223] Antibody [NBP1-78295] - WB analysis of BTKpY223 on Ramos whole cell lysate with peptide competition: 1. ab only, 2. 200 molar excess unmodified immunizing peptide and 3. 200 molar excess modified immunizing peptide.



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



Tolebrutinib treatment alleviates inflammatory response and ferroptosis in microglia. Tolebrutinib treatment alleviates inflammatory response and ferroptosis in microglia. (A) Experimental flow chart. (B) Primary microglia were treated with tolebrutinib at different concentrations for 4 h, and CCK8 were performed. One-way ANOVA and Tukey post hoc test.  $n = 6$  per group. (C-D) Representative image and quantification of pBTK protein levels in primary microglia. One-way ANOVA and Tukey post hoc test,  $n = 6$  per group. (E) Normalized mRNA levels of genes related to inflammatory response, phagocytosis and ferroptosis were measured. One-way ANOVA and Tukey post hoc test.  $n = 6$  per group. Image collected and cropped by CiteAb from the following open publication (<https://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-024-03187-4>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Zhang J FLT3/FLT3L signaling modulates splenic dendritic cells maturation upon traumatic brain injury and is enhanced by ethanol intoxication Thesis 2023-01-01 (ICC/IF, Mouse)

### Details:

1:200 ICC/IF dilution

Rehman R, Miller M, Krishnamurthy SS et al. Met/HGFR triggers detrimental reactive microglia in TBI Cell reports 2022-12-27 [PMID: 36577378] (IF/IHC, WB, Mouse)

Flümann R, Rehkemper T, Nieper P et al. An Autochthonous Mouse Model of Myd88- and BCL2-Driven Diffuse Large B-cell Lymphoma Reveals Actionable Molecular Vulnerabilities Blood Cancer Discov 2021-01-15 [PMID: 33447829] (PLA, Mouse)

### Details:

Citation using the HRP version of this antibody.

Wang SP, Iwata S, Nakayama S et al. Amplification of IL-21 signalling pathway through Bruton's tyrosine kinase in human B cell activation Rheumatology (Oxford) 2015-02-26 [PMID: 25724205] (FLOW, Human)

### Details:

BTK [p Tyr223] antibody used for FLOW on human CD19+ B cells in PBMCs alongwith rabbit IgG isotype control (see full text for detailed protocol).

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 2014-02-12 [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 2013-03-28 [PMID: 23539538] (WB, Human)



## Procedures

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

BTK [p Tyr223] Antibody:

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

BTK [p Tyr223] 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 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)**

BTK [p Tyr223] Antibody:  
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 1:25,000 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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### **Novus Biologicals USA**

10730 E. Briarwood Avenue  
Centennial, CO 80112  
USA  
Phone: 303.730.1950  
Toll Free: 1.888.506.6887  
Fax: 303.730.1966  
nb-customerservice@bio-techne.com

### **Bio-Techne Canada**

21 Canmotor Ave  
Toronto, ON M8Z 4E6  
Canada  
Phone: 905.827.6400  
Toll Free: 855.668.8722  
Fax: 905.827.6402  
canada.inquires@bio-techne.com

### **Bio-Techne Ltd**

19 Barton Lane  
Abingdon Science Park  
Abingdon, OX14 3NB, United Kingdom  
Phone: (44) (0) 1235 529449  
Free Phone: 0800 37 34 15  
Fax: (44) (0) 1235 533420  
info.EMEA@bio-techne.com

### **General Contact Information**

www.novusbio.com  
Technical Support: nb-technical@bio-techne.com  
Orders: nb-customerservice@bio-techne.com  
General: novus@novusbio.com

### **Products Related to NBP1-78295**

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NBP1-78295PEP	BTK [p Tyr223] Antibody Blocking Peptide
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

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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.

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