abcam

Product datasheet

Anti-HPRT antibody [EPR5299] ab109021





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Overview

Product name Anti-HPRT antibody [EPR5299]

Rabbit monoclonal [EPR5299] to HPRT **Description**

Host species Rabbit

Specificity The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for

mouse and rat.

Tested applications Suitable for: WB, IP, IHC-P

Unsuitable for: Flow Cyt or ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa HepG2, A549, and MCF7 cell lysates, Mouse brain and Rat brain tissue lysates IHC-

P:Human lung and colon cancer IP: Rat brain lysate

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol (glycerin, glycerine), 59% PBS, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number EPR5299

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab109021 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes	
WB		1/10000 - 1/50000. Predicted molecular weight: 25 kDa.	
IP		1/10 - 1/100.	
IHC-P		1/150. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. Heat up to 98°C, below boiling, and then let cool for 10-20 min. See IHC antigen retrieval protocols.	
		For unpurified use at 1/50 - 1/100	
		The mouse and rat recommendation is based on the WB results.	

Application notes

Is unsuitable for Flow Cyt or ICC/IF.

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Function Converts guanine to guanosine monophosphate, and hypoxanthine to inosine monophosphate.

Transfers the 5-phosphoribosyl group from 5-phosphoribosylpyrophosphate onto the purine. Plays

a central role in the generation of purine nucleotides through the purine salvage pathway.

Pathway Purine metabolism; IMP biosynthesis via salvage pathway; IMP from hypoxanthine: step 1/1.

Involvement in diseaseDefects in HPRT1 are the cause of Lesch-Nyhan syndrome (LNS) [MIM:300322]. LNS is

characterized by complete lack of enzymatic activity that results in hyperuricemia,

choreoathetosis, mental retardation, and compulsive self-mutilation.

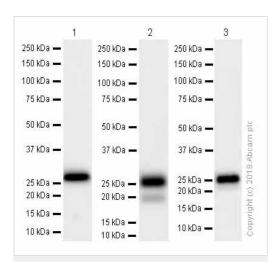
Defects in HPRT1 are the cause of gout HPRT-related (GOUT-HPRT) [MIM:300323]; also known as HPRT-related gout or Kelley-Seegmiller syndrome. Gout is characterized by partial enzyme

activity and hyperuricemia.

Sequence similaritiesBelongs to the purine/pyrimidine phosphoribosyltransferase family.

Cellular localization Cytoplasm.

Images



Western blot - Anti-HPRT antibody [EPR5299] (ab109021)

All lanes : Anti-HPRT antibody [EPR5299] (ab109021) at 0.02 µg/ml (purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lane 2 : Mouse brain lysates

Lane 3 : Rat brain lysates

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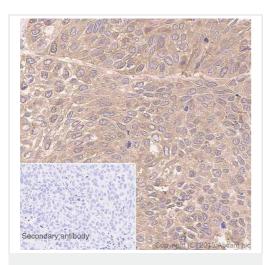
Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) ($\underline{ab97051}$) at 0.02 $\mu g/ml$

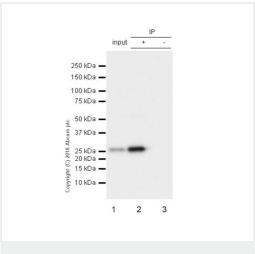
Predicted band size: 25 kDa

Blocking and diluting buffer: 5% NFDM/TBST.

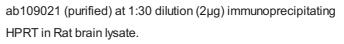


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HPRT antibody
[EPR5299] (ab109021)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human lung cancer tissue sections labeling HPRT with Purified ab109021 at 1:150 dilution (1.19 µg/ml). Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0)ImmunoHistoProbe one step HRP Polymer (ready to use)was used as the secondary antibody.Negative control:PBS instead of the primary antibody.Hematoxylinwas used as a counterstain



Immunoprecipitation - Anti-HPRT antibody [EPR5299] (ab109021)



Lane 1 (input): Rat brain lysate 10µg

Lane 2 (+): ab109021 & Rat brain lysate

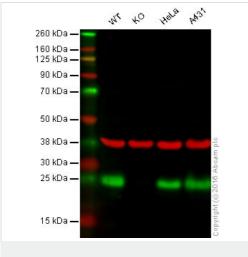
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of

ab109021 in Rat brain lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP)

(ab131366) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



Western blot - Anti-HPRT antibody [EPR5299] (ab109021)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)

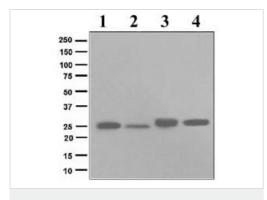
Lane 2: HPRT1 knockout HAP1 whole cell lysate (20 µg)

Lane 3: HeLa whole cell lysate (20 µg)

Lane 4: A431 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab109021 observed at 25 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

Unpurified ab109021 was shown to specifically react with HPRT1 in wild-type HAP1 cells. No band was observed when HPRT1 knockout samples were examined. Wild-type and HPRT1 knockout samples were subjected to SDS-PAGE. ab109021 and ab8245 (Mouse anti GAPDH loading control) were both diluted at 1/10,000 and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.



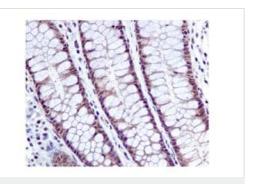
Western blot - Anti-HPRT antibody [EPR5299] (ab109021)

All lanes : Anti-HPRT antibody [EPR5299] (ab109021) at 1/10000 dilution (Unpurified)

Lane 1 : HeLa cell lysate
Lane 2 : HepG2 cell lysate
Lane 3 : A549 cell lysate
Lane 4 : MCF7 cell lysate

Lysates/proteins at 10 µg per lane.

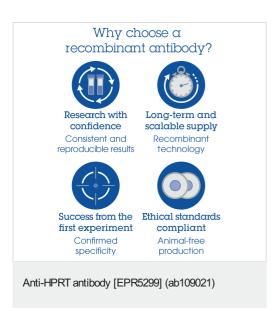
Predicted band size: 25 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HPRT antibody
[EPR5299] (ab109021)

Unpurified ab109021 at 1/50 dilution staining HPRT in Human colon by Immunohistochemistry, Paraffin-embedded tissue.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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