abcam

Product datasheet

Anti-phosphohistidine antibody ab231709

3 Images

Overview

Product name	Anti-phosphohistidine antibody
Description	Sheep polyclonal to phosphohistidine
Host species	Sheep
Tested applications	**Suitable for:** WB, IP, Competitive ELISA
Species reactivity	**Reacts with:** Species independent
Immunogen	Other Immunogen Type. A non-hydrolysable analogue of phosphohistidine, 1-(2-aminoethyl)pyrazol-4-ylphosphonic acid, conjugated to keyhole limpet hemocyanin (KLH) via a glutaraldehyde linkage.
Positive control	Competitive ELISA: Phosphorylated amino acids and His, using t-pHis conjugated to BSA. WB: Human bronchial epithelial cells. IP: pHis proteins from human bronchial epithelial cells.
General notes	It is recommended the antibody is centrifuged at 11200 RCF at 4°C for 10 minutes before use and supernatant used for analysis. The following buffers and conditions for the Western blot are suggested: Lysis buffer: 150 mM sodium chloride, 0.5 % sodium deoxycholate, 1% Triton X-100, 0.1 % sodium dodecyl sulfate, 10 mM sodium fluoride, 5 mM sodium orthovanadate, 10 mM sodium pyrophosphate, protease inhibitor cocktail, 50 mM tris base, pH 9.0. Denature samples in 5x loading buffer with; 10 % lithium dodecyl sulphate, 40% glycerol, 0.02% bromophenol blue, 50 mM ethylenediaminetetraacetic acid disodium salt dihydrate, 500 mM dithiothreitol, 300 mM tris base, pH 8.8 for 30 min at RT (do not heat). Block in; 10 mM tris-hydrochloride, 165 mM sodium chloride, pH 8.0, 0.05 % (v/v), Tween 20, 0.2 % (v/v) freshwater fish gelatin at RT for 1 hr. As a control phosphohistidine can be reduced/abolished by reducing the pH (pH 2-4) in denaturing buffer and then heating between 60-90 ºC for > 30 min.

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: 7.40

	Preservative: 0.05% Sodium azide Constituents: Tris glycine, 0.87% Sodium chloride
Purity	Affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

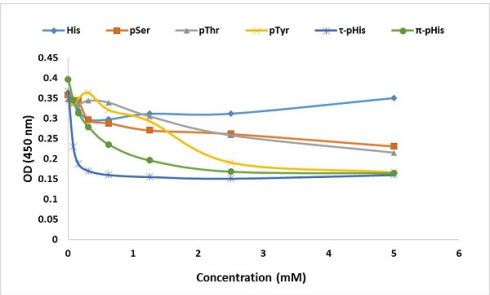
Our Abpromise guarantee covers the use of **ab231709** in the following tested applications.

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

Application	Abreviews	Notes
WB		1/4000.
IP		Use at an assay dependent concentration.
Competitive ELISA		Use at an assay dependent concentration.

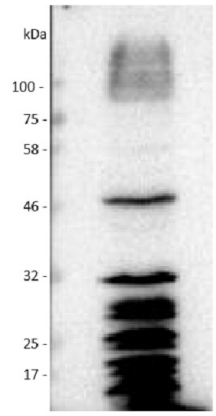
Target

Images



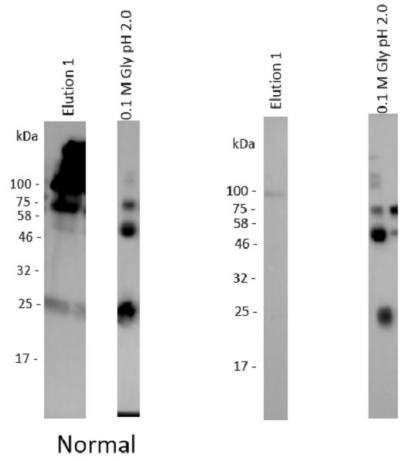
Competitive ELISA - Anti-phosphohistidine antibody (ab231709)

Competitive ELISA of ab231709 against phosphorylated amino acids and His, using τ-pHis conjugated to BSA.



Western blot - Anti-phosphohistidine antibody (ab231709)

Anti-phosphohistidine antibody (ab231709) at 1/4000 dilution + Human bronchial epithelial cell lysate at 100 µg



Immunoprecipitation - Anti-phosphohistidine antibody (ab231709)

Immunoprecipitation of pHis proteins from human bronchial epithelial cells using ab231709. Western blot of immunoprecipitates using ab231709 at 1/4000 dilution.

pHis antibodies are immobilised using protein G sepharose. Elution refers to batch extraction with competitor. pHis antibody used to probe proteins on PVDF membrane.

Right pair of images: pHis is known to be acid labile: Acid treatment (pH 7, 60ºC 30 minutes) of lysate before IP.

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