abcam

Product datasheet

Anti-USP9x antibody ab19879

敲除验证

★★★★★ 1 Abreviews 17 References 6 图像

概述

产品名称	Anti-USP9x抗体
描述	兔多克隆抗体to USP9x
宿主	Rabbit
特异性	ab19879 detects 289kDa full length USP9X Human protein (Q93008) in WB on Caco2 Lysate. All detected bands are quenched by the immunizing peptide **ab20617**
经测试应用	**适用于:** ICC/IF, IP, ICC, IHC-Fr, WB, IHC (PFA fixed)
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide conjugated to KLH derived from within residues 1 - 100 of Human USP9x. 参阅Abcam的专有抗源政策
阳性对照	ICC: Hek293 cells
常规说明	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

纯度	Immunogen affinity purified
克隆	**多克隆**
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab19879于以下的经测试应用**

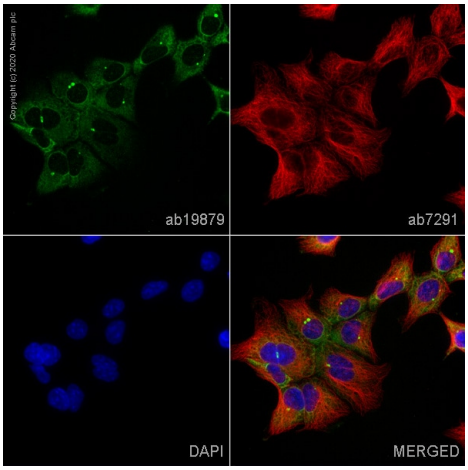
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
ICC		Use a concentration of 5 µg/ml.
IHC-Fr	★★★★★ (1)	Use at an assay dependent concentration.
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 100,105, 290 kDa (predicted molecular weight: 54, 100,105 , 290 kDa).
IHC (PFA fixed)		Use a concentration of 0.1 µg/ml.

靶标

功能	Deubiquitinase involved both in the processing of ubiquitin precursors and of ubiquitinated proteins. May therefore play an important role regulatory role at the level of protein turnover by preventing degradation of proteins through the removal of conjugated ubiquitin. Essential component of TGF-beta/BMP signaling cascade. Regulates chromosome alignment and segregation in mitosis by regulating the localization of BIRC5/survivin to mitotic centromeres. Specifically hydrolyzes both 'Lys-29'- and 'Lys-33'-linked polyubiquitins chains. Specifically deubiquitinates monoubiquitinated SMAD4, opposing the activity of E3 ubiquitin-protein ligase TRIM33.
组织特异性	Widely expressed in embryonic and adult tissues.
序列相似性	Belongs to the peptidase C19 family.
细胞定位	Cytoplasm.

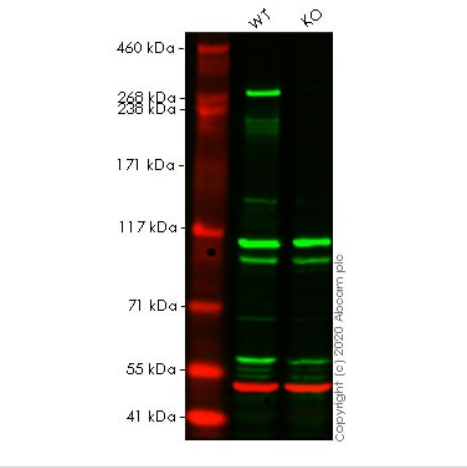
图片



Immunocytochemistry - Anti-USP9x antibody (ab19879)

ab19879 staining USP9x in Hek293 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab19879 at 1µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue). Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Western blot - Anti-USP9x antibody (ab19879)

All lanes : Anti-USP9x antibody (ab19879) at 1 µg/ml

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : USP9X knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

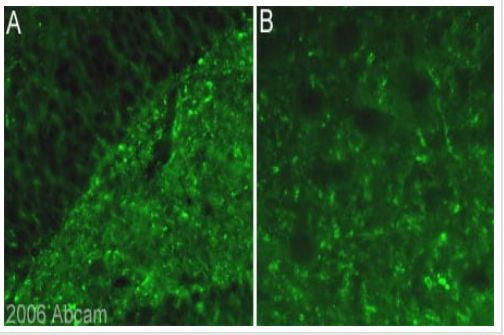
Performed under reducing conditions.

Predicted band size: 54, 100,105 , 290 kDa
Observed band size: 290 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab19879 observed at 290 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab19879 was shown to react with USP9x in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab265665** (knockout cell lysate **ab257790**) was used. Wild-type HeLa and USP9X knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room

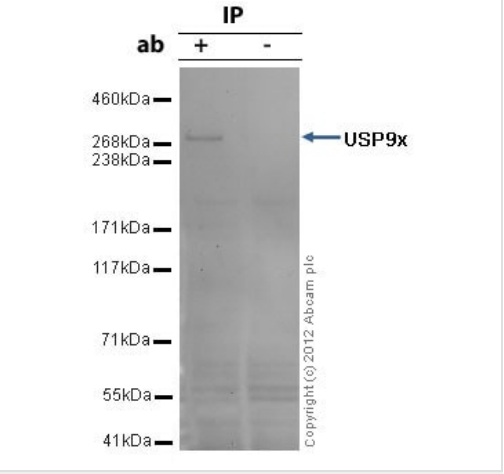
temperature in 0.1% TBST with 3% non-fat dried milk. ab19879 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) were incubated overnight at 4°C at a 1 µg/ml and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (PFA fixed) - Anti-USP9x antibody (ab19879)

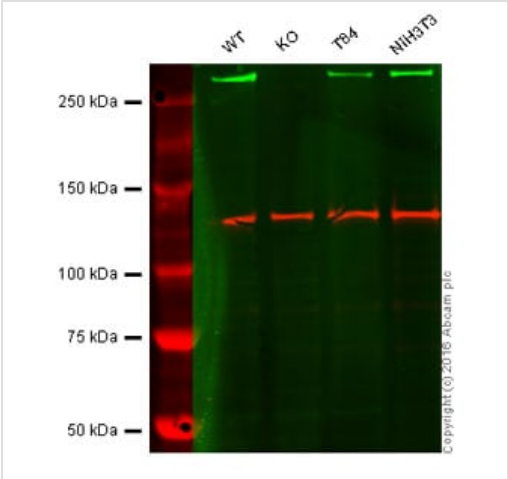
This image is courtesy of Sophie Pezet, King's College London, United Kingdom

Immuofluorescent staining for USP9X in the rat hippocampus (dentate gyrus) using ab19879 (1/300 = 0.07µg/ml). Image is taken with X10 objective. ab19879 was incubated overnight at RT. Secondary antibody used was anti-rabbit Alexa fluor 488 (1/1000 for 2h at RT). Rats were intracardially perfused with paraformaldehyde 4%, brain tissue was post-fixed overnight in the same fixative, cryoprotected in 20% sucrose and frozen in OCT. 30µm coronal sections were cut on a cryostat for free floating IHC.



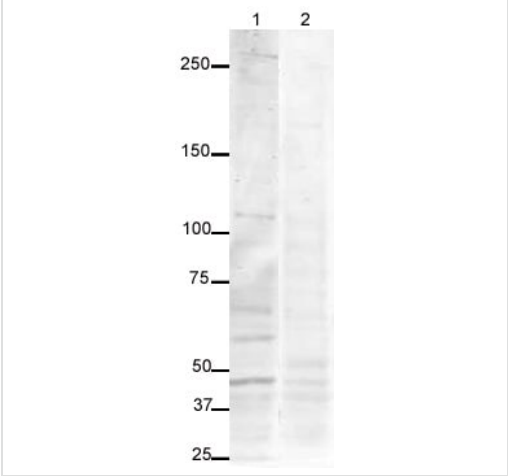
Immunoprecipitation - Anti-USP9x antibody (ab19879)

USP9x was immunoprecipitated using 0.5mg Caco2 whole cell extract, 5µg of Rabbit polyclonal to USP9x and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, Caco2 whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab19879.
Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (**ab99697**).
Band: 290kDa: USP9x.



Western blot - Anti-USP9x antibody (ab19879)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: USP9x knockout HAP1 cell lysate (20 µg)
Lane 3: T84 cell lysate (20 µg)
Lane 4: NIH3T3 cell lysate (20 µg)
Lanes 1 to 4: Merged signal (red and green). Green - ab19879 observed at 290 kDa. Red - loading control, **ab181602** observed at 124 kDa.
ab19879 was shown to specifically react with USP9x when USP9x knockout samples were used. Wild-type and USP9x knockout samples were subjected to SDS-PAGE. **ab9879** and **ab181602** (loading control to GAPDH) were both diluted at 1 µg/ml and 1/10000 respectively and incubated overnight at 4°C. Blots were developed withGoat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-USP9x antibody (ab19879)

All lanes : Anti-USP9x antibody (ab19879) at 1 µg/ml

Lane 1 : Caco-2 whole cell lysate (**ab3950**)
Lane 2 : Caco-2 whole cell lysate (**ab3950**) with Human USP9x peptide (**ab20617**) at 1 µg/ml

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Alexa Fluor Goat polyclonal to Rabbit IgG (700) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 54, 100,105 , 290 kDa
Observed band size: 100,105,290 kDa
Additional bands at: ~50-65 kDa. We are unsure as to the identity of these extra bands.

ab19879 detects full length USP9x protein as well as a number of USP9x fragments in WB on Caco2 Lysate:

289kDa Human protein: Q93008 USP9X (Full length protein)
105kDa Human protein: Q6P468 - USP9X protein (Fragment)
Human protein
99.7kDa Human protein: Q59EZ5 - USP9X protein variant (Fragment).
53.9kDa Q86X58 - USP9X protein (Fragment).

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