abcam

Product datasheet

Anti-RBX1 antibody [EPR20185] ab221548

Recombinant RabMAb

15 Images

Overview

Product name	Anti-RBX1 antibody [EPR20185]
Description	Rabbit monoclonal [EPR20185] to RBX1
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, Flow Cyt, IP, ICC/IF, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human RBX1 aa 50 to the C-terminus. The exact sequence is proprietary. Database link: P62877
Positive control	WB: HeLa, HepG2, HT-1080, HEK-293, RAW 264.7, PC-12 and NIH/3T3 whole cell lysates; Human fetal heart and fetal kidney lysates; Mouse heart, kidney and spleen lysates; Rat brain, kidney and spleen lysates. IHC-P: Human colon, colon carcinoma, lung, lung carcinoma, gastric carcinoma and bladder cancer tissues; Mouse stomach tissue; Rat colon tissue. ICC/IF: HeLa and NIH/3T3 cells. Flow Cyt: HeLa and NIH/3T3 cells. IP: HeLa whole cell lysate.
General notes	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents. This product is a recombinant rabbit monoclonal antibody.

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	Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol, PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20185
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab221548** in the following tested applications.

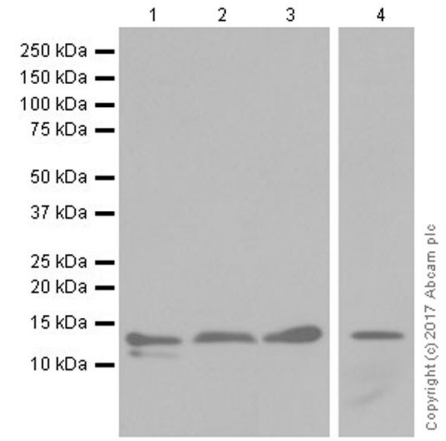
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt		1/60.
IP		1/40.
ICC/IF		1/500.
WB		1/1000. Detects a band of approximately 12,11 kDa (predicted molecular weight: 12 kDa).

Target

Function	E3 ubiquitin ligase component of multiple cullin-RING-based E3 ubiquitin-protein ligase complexes which mediate the ubiquitination and subsequent proteasomal degradation of target proteins, including proteins involved in cell cycle progression, signal transduction, transcription and transcription-coupled nucleotide excision repair. The functional specificity of the E3 ubiquitin-protein ligase complexes depends on the variable substrate recognition components. As a component of the CSA complex promotes the ubiquitination of ERCC6 resulting in proteasomal degradation. Through the RING-type zinc finger, seems to recruit the E2 ubiquitination enzyme, like CDC34, to the complex and brings it into close proximity to the substrate. Probably also stimulates CDC34 autoubiquitination. May be required for histone H3 and histone H4 ubiquitination in response to ultraviolet and for subsequent DNA repair. Promotes the neddylation of CUL1, CUL2, CUL4 and CUL4 via its interaction with UBE2M.
Tissue specificity	Widely expressed.
Pathway	Protein modification; protein ubiquitination.
Sequence similarities	Belongs to the RING-box family. Contains 1 RING-type zinc finger.
Domain	The RING-type zinc finger domain is essential for ubiquitin ligase activity. It coordinates an additional third zinc ion.
Cellular localization	Cytoplasm. Nucleus.

Images



Western blot - Anti-RBX1 antibody [EPR20185] (ab221548)

All lanes : Anti-RBX1 antibody [EPR20185] (ab221548) at 1/5000 dilution

Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate
Lane 3 : HT1080 (human fibrosarcoma cell line) whole cell lysate
Lane 4 : HEK-293 (human epithelial cell line from embryonic kidney) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

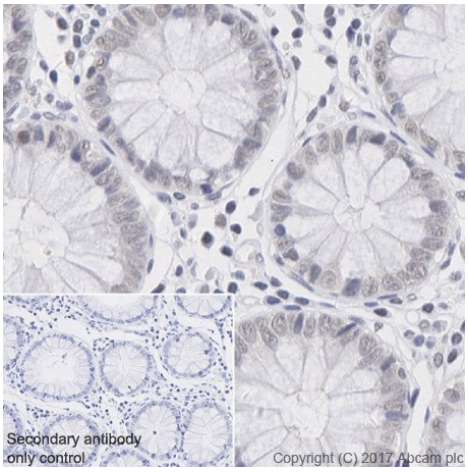
Developed using the ECL technique.

Predicted band size: 12 kDa
Observed band size: 11,12 kDa
why is the actual band size different from the predicted?

Exposure time : Lanes 1-3: 30 seconds; Lane 4: 10 seconds.

Blocking/Dilution buffer: 5% NFDM/TBST.

The molecular weight observed is consistent with what has been described in the literature (PMID: 22822056).



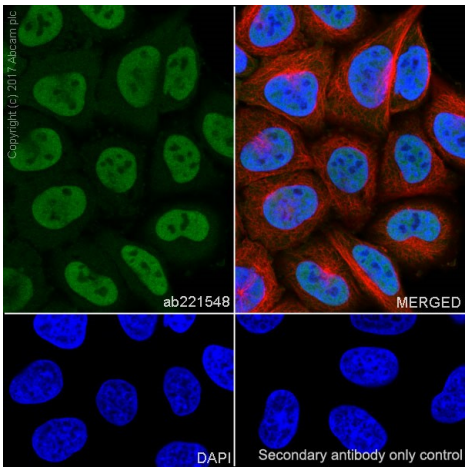
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RBX1 antibody [EPR20185] (ab221548)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling RBX1 with ab221548 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear staining is observed on human colon tissue section.

As documented in the literature RBX1 has lower expression level in normal tissue compared to cancerous tissue (PMID:19509229).

Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

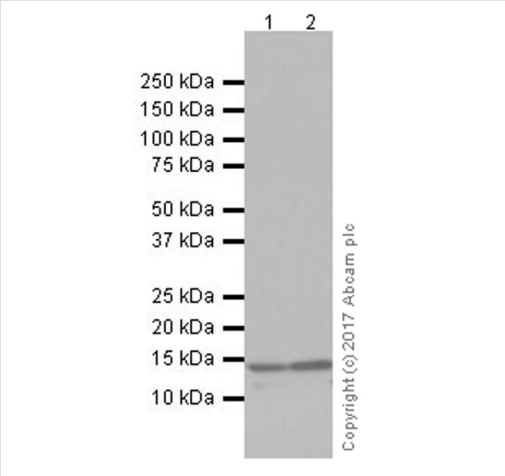


Immunocytochemistry/ Immunofluorescence - Anti-RBX1 antibody [EPR20185] (ab221548)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling RBX1 with ab221548 at 1/500 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear and weak cytoplasmic staining on HeLa cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.



Western blot - Anti-RBX1 antibody [EPR20185] (ab221548)

All lanes : Anti-RBX1 antibody [EPR20185] (ab221548) at 1/1000 dilution

Lane 1 : Human fetal heart lysate
Lane 2 : Human fetal kidney lysate

Lysates/proteins at 20 µg per lane.

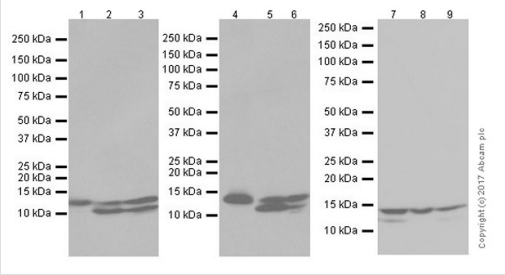
Secondary
All lanes : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/4000 dilution

Developed using the ECL technique.

Predicted band size: 12 kDa
Observed band size: 12 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-RBX1 antibody [EPR20185] (ab221548)

All lanes : Anti-RBX1 antibody [EPR20185] (ab221548) at 1/1000 dilution

Lane 1 : Mouse heart lysate
Lane 2 : Mouse kidney lysate
Lane 3 : Mouse spleen lysate
Lane 4 : Rat brain lysate
Lane 5 : Rat kidney lysate
Lane 6 : Rat spleen lysate
Lane 7 : RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate
Lane 8 : PC-12 (rat adrenal gland pheochromocytoma cell line) whole cell lysate
Lane 9 : NIH/3T3 (mouse embyro fibroblast cell line) whole cell

lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

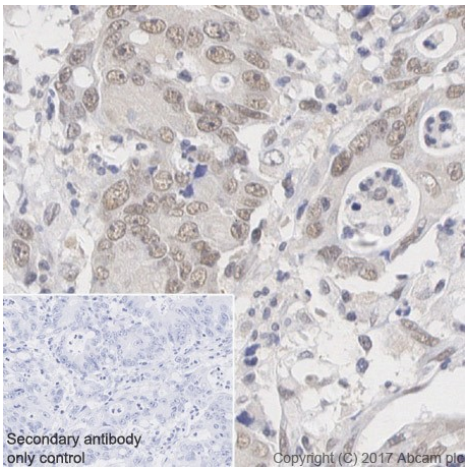
Developed using the ECL technique.

Predicted band size: 12 kDa
Observed band size: 11,12 kDa why is the actual band size different from the predicted?

Exposure time : Lanes 1-6: 3 seconds; Lanes 7-8: 5 seconds.

Blocking/Dilution buffer: 5% NFDM/TBST.

The molecular weight observed is consistent with what has been described in the literature (PMID: 22822056).



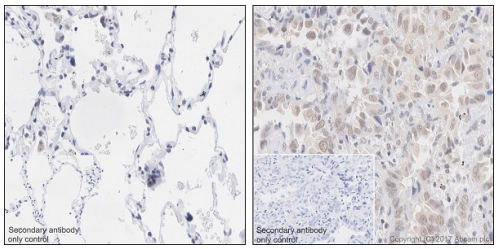
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RBX1 antibody [EPR20185] (ab221548)

Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue labeling RBX1 with ab221548 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear and cytoplasmic staining is observed on human colon carcinoma tissue sections.

As documented in the literature RBX1 has higher expression level in cancerous tissue compared to normal tissue (PMID:19509229).

Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.



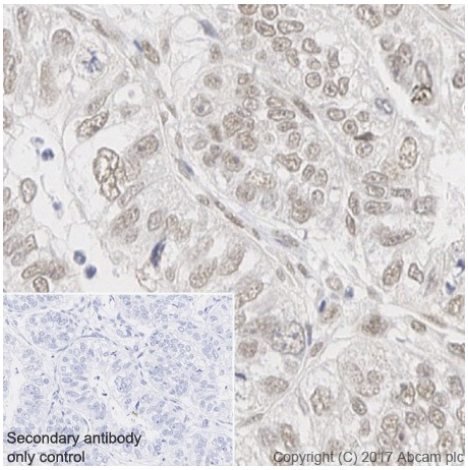
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RBX1 antibody [EPR20185] (ab221548)

Immunohistochemical analysis of paraffin-embedded human lung and lung carcinoma tissues labeling RBX1 with ab221548 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Weak nuclear staining of RBX1 on the epithelium cells of human lung (left) compared to strong staining in human lung carcinoma (right).

As documented in the literature ROC1 has higher expression level in cancerous tissue compared to normal tissue (PMID:19509229).

Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

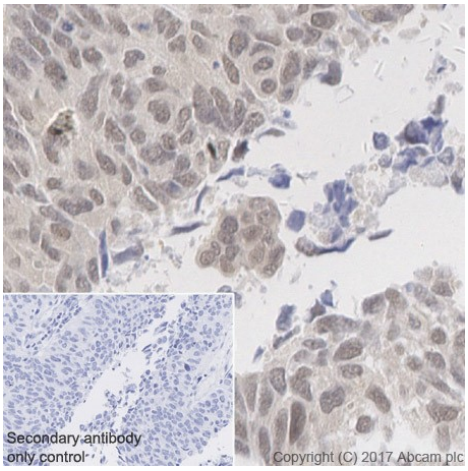


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RBX1 antibody [EPR20185] (ab221548)

Immunohistochemical analysis of paraffin-embedded human gastric carcinoma tissue labeling RBX1 with ab221548 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear and cytoplasmic staining on human gastric carcinoma tissue sections. The staining pattern observed is consistent with what has been described in the literature (PMID:24292229).

Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

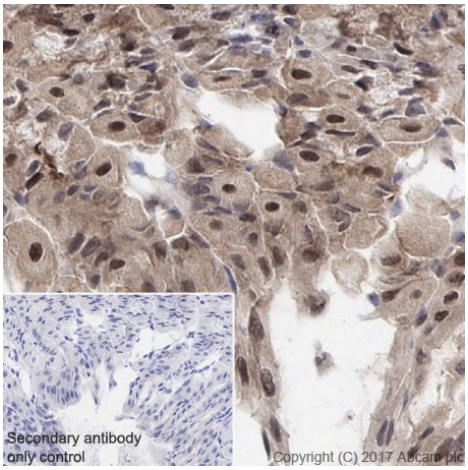


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RBX1 antibody [EPR20185] (ab221548)

Immunohistochemical analysis of paraffin-embedded human bladder cancer tissue labeling RBX1 with ab221548 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear and cytoplasmic staining on human bladder carcinoma tissue sections. The staining pattern observed is consistent with what has been described in the literature (PMID:23667514).

Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

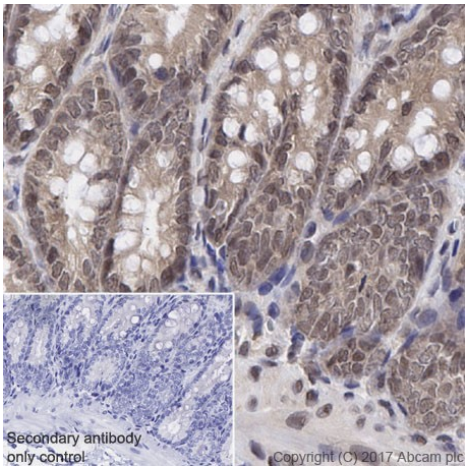


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RBX1 antibody [EPR20185] (ab221548)

Immunohistochemical analysis of paraffin-embedded mouse stomach tissue labeling RBX1 with ab221548 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear and cytoplasmic staining on mouse stomach tissue sections.

Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

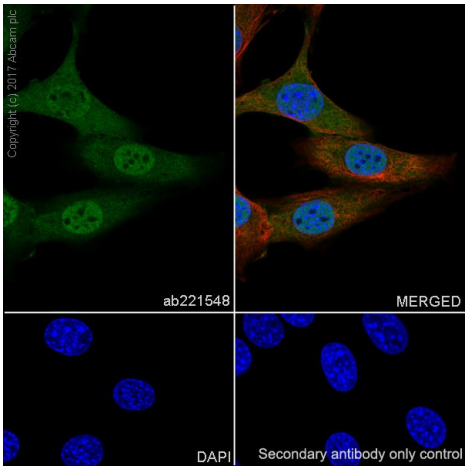


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RBX1 antibody [EPR20185] (ab221548)

Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling RBX1 with ab221548 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear and cytoplasmic staining on rat colon tissue sections.

Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

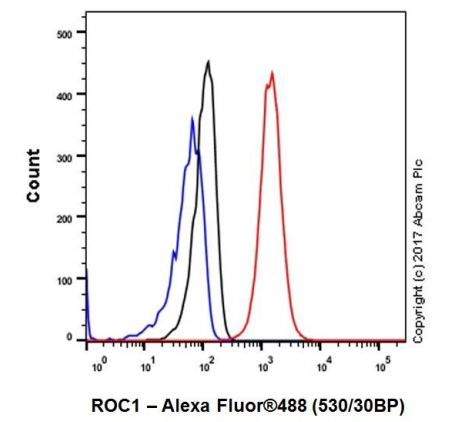


Immunocytochemistry/ Immunofluorescence - Anti-RBX1 antibody [EPR20185] (ab221548)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (mouse embyro fibroblast cell line) cells labeling RBX1 with ab221548 at 1/500 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear and weak cytoplasmic staining on NIH/3T3 cell line.

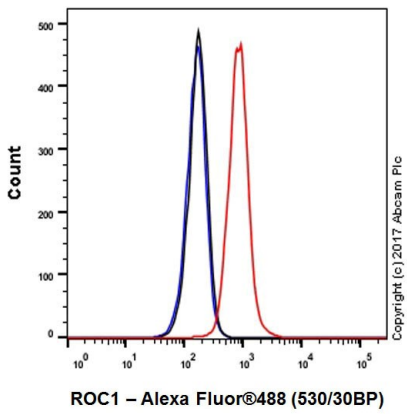
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.



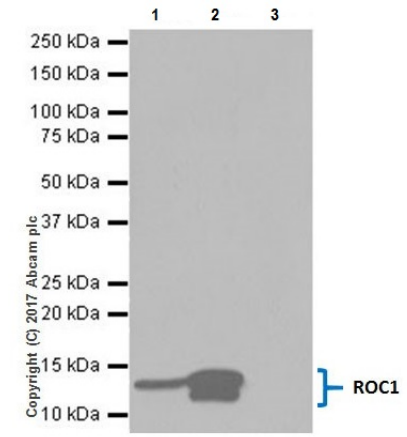
Flow Cytometry - Anti-RBX1 antibody [EPR20185] (ab221548)

Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cell line labeling RBX1 with ab221548 at 1/600 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry - Anti-RBX1 antibody [EPR20185] (ab221548)

Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized NIH/3T3 (mouse embyro fibroblast cell line) cell line labeling RBX1 with ab221548 at 1/60 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-RBX1 antibody [EPR20185] (ab221548)

RBX1 was immunoprecipitated from 0.35 mg of HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab221548 at 1/40 dilution. Western blot was performed from the immunoprecipitate using ab221548 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

Lane 1: HeLa whole cell lysate 10 µg (Input).

Lane 2: ab221548 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab221548 in HeLa whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 30 seconds.

The molecular weight observed is consistent with what has been described in the literature (PMID: 22822056).

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours

- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors