abcam

ab134953

Anti-Ubiquitin antibody [EPR8830]

Anti-Ubiquitin antibody [EPR8830] (ab134953) is a rabbit monoclonal antibody detecting Ubiquitin in **Western Blot, Flow Cytometry (Intra), IHC-P, ICC/IF**. Suitable for **Human, Mouse, Rat**.

- Biophysical QC for unrivalled batch-batch consistency
- Over 100 publications

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR8830
Purification technique	Affinity purification Protein A
Concentration	0.7 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Mouse
Dilution info	1/1000 - 1/10000
Notes	Monoubiquitin molecular weight

Species	Rat
Dilution info	1/1000 - 1/10000
Notes	Monoubiquitin molecular weight

Species	Human
Dilution info	1/1000 - 1/10000
Notes	Monoubiquitin molecular weight

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	**For unpurified use at 1/250 - 1/500.**

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/70
Notes	For unpurified use at 1/100 - 1/1000. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	1/800
Notes	**For unpurified use at 1/250 - 1/500.**

Species	Rat
Dilution info	1/800
Notes	**For unpurified use at 1/250 - 1/500.**

Species	Human
Dilution info	1/800
Notes	**For unpurified use at 1/250 - 1/500.**

Target data

See full target information UBB

Function

Ubiquitin. Exists either covalently attached to another protein, or free (unanchored). When covalently bound, it is conjugated to target proteins via an isopeptide bond either as a monomer (monoubiquitin), a polymer linked via different Lys residues of the ubiquitin (polyubiquitin chains) or a linear polymer linked via the initiator Met of the ubiquitin (linear polyubiquitin chains). Polyubiquitin chains, when attached to a target protein, have different functions depending on the Lys residue of the ubiquitin that is linked: Lys-6-linked may be involved in DNA repair; Lys-11-linked is involved in ERAD (endoplasmic reticulum-associated degradation) and in cell-cycle regulation; Lys-29-linked is involved in proteotoxic stress response and cell cycle; Lys-33-linked is involved in kinase modification; Lys-48-linked is involved in protein degradation via the proteasome; Lys-63-linked is involved in endocytosis, DNA-damage responses as well as in signaling processes leading to activation of the transcription factor NF-kappa-B. Linear polymer chains formed via attachment by the initiator Met lead to cell signaling. Ubiquitin is usually conjugated to Lys residues of target proteins, however, in rare cases, conjugation to Cys or Ser residues has been observed. When polyubiquitin is free (unanchored-polyubiquitin), it also has distinct roles, such as in activation of protein kinases, and in signaling.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle, Stable for 12 months at -20°C

Notes

Product Specifications
Anti-Ubiquitin antibody [EPR8830] (ab134953) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IHC-P, WB in human, mouse, rat samples.
Anti-Ubiquitin antibody [EPR8830] (ab134953) specifically detects Ubiquitin (UniProt ID: P0CG47; Molecular weight: 9kDa) and is sold in a convenient trial size to enable initial testing (20 µL) and larger sizes for subsequent scaling up experiments (100 µL and 1 mL).

Quality and Validation
Abcam's high quality manufacturing and validation processes ensure Anti-Ubiquitin antibody [EPR8830] (ab134953) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.
Anti-Ubiquitin antibody [EPR8830] (ab134953) has been cited over 105 times in peer reviewed journals and is trusted by the scientific community.

Related Products
Antibody clone EPR8830 is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 488, Alexa Fluor® 647, PE, APC, HRP, Alexa Fluor® 568, Alkaline Phosphatase, Alexa Fluor® 594, Alexa Fluor® 555, Alexa Fluor® 750 (ab205467, ab205468, ab303020, ab303021, ab303022, ab307636, ab308798, ab310519, ab312049, ab321094).
Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

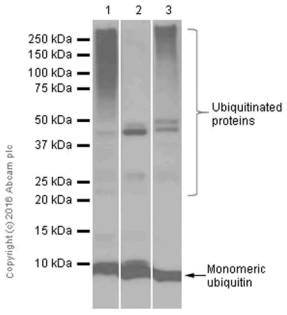
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

16 product images



Western blot - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Blocking and diluting buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-Ubiquitin antibody [EPR8830] (ab134953) at 0.7 µg/mL

Lane 1:
Rat brain lysate at 20 µg

Lane 2:
Mouse kidney lysate at 20 µg

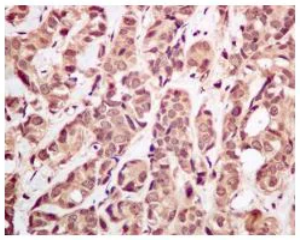
Lane 3:
Rat kidney lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 26 kDa

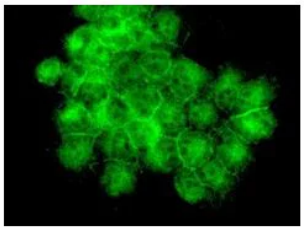
Observed band size: 8 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Ubiquitin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Ubiquitin antibody

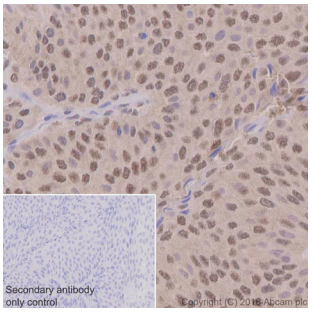
Immunohistochemical analysis of paraffin embedded Human breast carcinoma tissue labelling Ubiquitin with ab134953 at 1/250 dilution



Immunocytochemistry/ Immunofluorescence - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Ubiquitin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Ubiquitin antibody

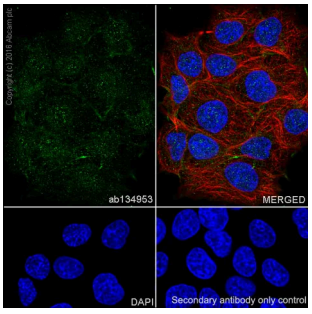
Immunofluorescent staining of JAR cells labelling Ubiquitin using ab134953 at 1/250 dilution



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Ubiquitin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Ubiquitin antibody

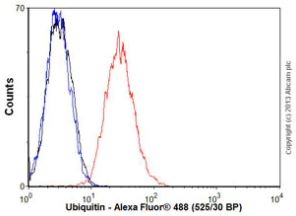
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human bladder carcinoma tissue sections labeling Ubiquitin with Purified ab134953 at 1:800 dilution (0.9 µg/ml). Heat mediated antigen retrieval was performed using EDTA Buffer, pH 9.0. Tissue was counterstained with Hematoxylin. ab97051 Goat Anti-Rabbit IgG H&L (HRP) secondary antibody was used at 1:500 dilution. PBS instead of the primary antibody was used as the negative control.



Immunocytochemistry/ Immunofluorescence - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Ubiquitin Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Ubiquitin antibody

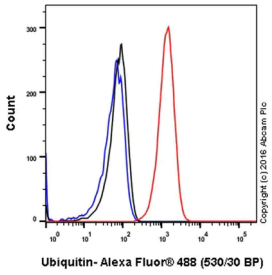
Immunocytochemistry/ Immunofluorescence analysis of JAR (Human placenta choriocarcinoma cell line) cells labeling Ubiquitin with Purified ab134953 at 1:100 dilution (7.2µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). ab150077 Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Flow Cytometry (Intracellular) - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Ubiquitin Flow Cytometry (Intracellular) staining using rabbit Anti-Ubiquitin antibody

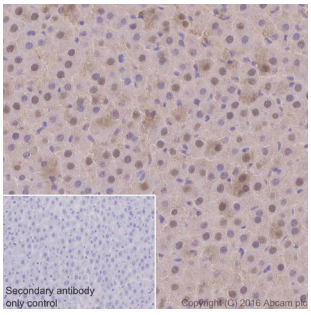
Overlay histogram showing HepG2 cells stained with ab134953 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab134953, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in HepG2 cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Flow Cytometry (Intracellular) - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Ubiquitin Flow Cytometry (Intracellular) staining using rabbit Anti-Ubiquitin antibody

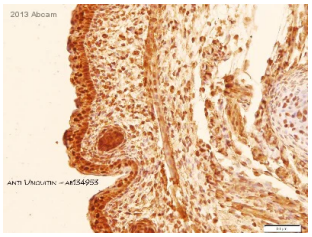
Intracellular Flow Cytometry analysis of JAR (Human placenta choriocarcinoma cell line) cells labeling Ubiquitin with purified ab134953 at 1/70 dilution (10 ug/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Ubiquitin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Ubiquitin antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Rat liver tissue sections labeling Ubiquitin with Purified ab134953 at 1:800 dilution (0.9 µg/ml). Heat mediated antigen retrieval was performed using EDTA Buffer, pH 9.0. Tissue was counterstained with Hematoxylin. ab97051 Goat Anti-Rabbit IgG H&L (HRP)
secondary antibody was used at 1:500 dilution. PBS instead of the primary antibody was used as the negative control.

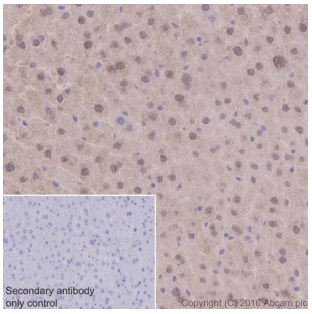


This image is courtesy of an anonymous customer review.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Ubiquitin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Ubiquitin antibody

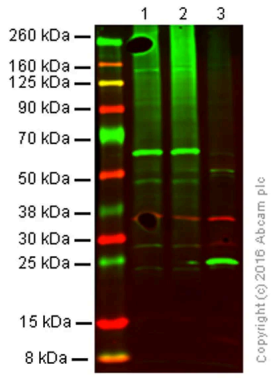
ab134953 staining Ubiquitin in Mouse embryo tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 1% FBS/BSA for 1 hour at room temperature; antigen retrieval was by heat mediation in citrate buffer, pH6. Samples were incubated with primary antibody (1/100 in 1% FBS/BSA) for 16 hours at 4°C. An undiluted HRP-conjugated Goat anti-rabbit IgG polyclonal was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Ubiquitin Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Ubiquitin antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Mouse liver tissue sections labeling Ubiquitin with Purified ab134953 at 1:800 dilution (0.9 µg/ml). Heat mediated antigen retrieval was performed using EDTA Buffer, pH 9.0. Tissue was counterstained with Hematoxylin. ab97051 Goat Anti-Rabbit IgG H&L (HRP)
secondary antibody was used at 1:500 dilution. PBS instead of the primary antibody was used as the negative control.



Western blot - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Ubiquitin Western blot staining using rabbit Anti-Ubiquitin antibody

This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a nitrocellulose membrane at 30V for 70 minutes .ab134953 and ab8245 (loading control to GAPDH) were diluted 1/200 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with goat anti-rabbit IgG (H + L) and goat anti-mouse IgG (H + L) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging using the Licor Odyssey CLx.
All lanes:
Western blot - Anti-Ubiquitin antibody [EPR8830] (ab134953) at 1/200 dilution

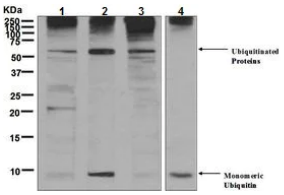
Lane 1:
MCF-7 Whole cell lysate at 20 µg

Lane 2:
MCF-7 Whole cell lysate + MG-132 (50 uM 90 min) at 20 µg

Lane 3:
Mouse Brain at 20 µg

Performed under reducing conditions.

Predicted band size: 26 kDa



Western blot - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Note: Ubiquitin exists as monomeric ubiquitin (8 kDa) or attached to other proteins.
All lanes:
Western blot - Anti-Ubiquitin antibody [EPR8830] (ab134953) at 1/1000 dilution

Lane 1:
293T cell lysate at 10 µg

Lane 2:
HepG2 cell lysate at 10 µg

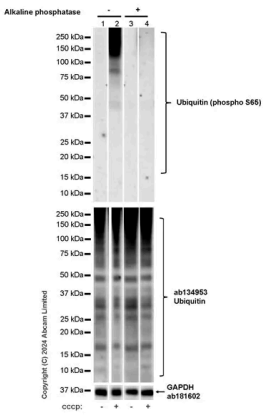
Lane 3:
HeLa cell lysate at 10 µg

Lane 4:
JAR cell lysate at 10 µg

Secondary

All lanes:
HRP labelled goat anti rabbit at 1/2000 dilution

Predicted band size: 26 kDa



Western blot - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Ubiquitin Western blot staining using rabbit Anti-Ubiquitin antibody

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

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

In Western blot, Anti-Ubiquitin antibody [EPR8830] (ab134953) staining at 1/1000 dilution.

All lanes:
Western blot - Anti-Ubiquitin (phospho S65) antibody [30H3/30K1] - Rat IgG2a (Chimeric) (ab320096) at 1/1000 dilution

Lane 1:
Untreated PC-3 (human prostate adenocarcinoma epithelial cell) whole cell lysate (untreated membrane) at 20 µg

Lane 2:
PC-3 treated with 30 µM CCCP (ab141229) for 2 hours, whole cell lysate (untreated membrane) at 20 µg

Lane 3:
Untreated PC-3 (human prostate adenocarcinoma epithelial cell) whole cell lysate (Alkaline phosphatase treated membrane) at 20 µg

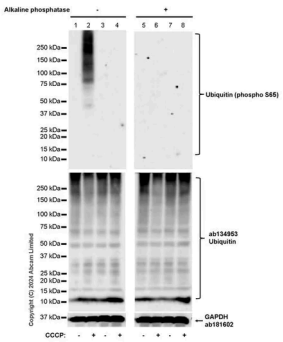
Lane 4:
PC-3 treated with 30 µM CCCP (ab141229) for 2 hours, whole cell lysate (Alkaline phosphatase treated membrane) at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rat IgG H&L (HRP) (ab205720) at 1/10000 dilution

Observed band size: 8-200 kDa, 36 kDa

Exposure time: 180s



Western blot - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Ubiquitin Western blot staining using rabbit Anti-Ubiquitin antibody

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

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

In Western blot, Anti-Ubiquitin antibody [EPR8830] (ab134953) staining at 1/1000 dilution.

All lanes:
Western blot - Anti-Ubiquitin (phospho S65) antibody [30H3/30K1] - Rat IgG2a (Chimeric) (ab320096) at 1/1000 dilution

Lane 1:
Untreated Wild-type 293T (human embryonic kidney epithelial cell) whole cell lysate (untreated membrane) at 20 µg

Lane 2:
293T treated with 20 µM CCCP (ab141229) for 8 hours, whole cell lysate (untreated membrane) at 20 µg

Lane 3:
Untreated PINK1 knockout HEK-293T whole cell lysate (untreated membrane) at 20 µg

Lane 4:
PINK1 knockout HEK-293T treated with 20 µM CCCP (ab141229) for 8 hours, whole cell lysate (untreated membrane) at 20 µg

Lane 5:
Untreated Wild-type 293T (human embryonic kidney epithelial cell) whole cell lysate (Alkaline phosphatase treated membrane) at 20 µg

Lane 6:
293T treated with 20 µM CCCP (ab141229) for 8 hours, whole cell lysate (Alkaline phosphatase treated membrane) at 20 µg

Lane 7:
Untreated PINK1 knockout HEK-293T whole cell lysate (Alkaline phosphatase treated membrane) at 20 µg

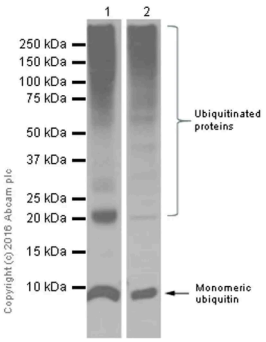
Lane 8:
PINK1 knockout HEK-293T treated with 20 µM CCCP (ab141229) for 8 hours, whole cell lysate (Alkaline phosphatase treated membrane) at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rat IgG H&L (HRP) (ab205720) at 1/10000 dilution

Observed band size: 8-200 kDa, 36 kDa

Exposure time: 92s



Western blot - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Blocking and diluting buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-Ubiquitin antibody [EPR8830] (ab134953) at 0.1 µg/mL

Lane 1:
JAR (Human placenta choriocarcinoma epithelial cell) whole cell lysate at 20 µg

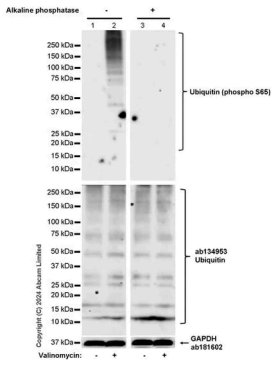
Lane 2:
293 (Human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 26 kDa

Observed band size: 8 kDa



Western blot - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Ubiquitin Western blot staining using rabbit Anti-Ubiquitin antibody

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

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution.

In Western blot, Anti-Ubiquitin antibody [EPR8830] (ab134953) staining at 1/1000 dilution.

All lanes:
Western blot - Anti-Ubiquitin (phospho S65) antibody [30H3/30K1] - Rat IgG2a (Chimeric) (ab320096) at 1/1000 dilution

Lane 1:
Untreated MEF (mouse embryo fibroblast) whole cell lysate (untreated membrane) at 20 µg

Lane 2:
MEF treated with 5 µM Valinomycin for 24 hours whole cell lysate (untreated membrane) at 20 µg

Lane 3:
Untreated MEF (mouse embryo fibroblast) whole cell lysate (Alkaline phosphatase treated membrane) at 20 µg

Lane 4:
MEF treated with 5 µM Valinomycin for 24 hours whole cell lysate (Alkaline phosphatase treated membrane) at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rat IgG H&L (HRP) (ab205720) at 1/10000 dilution

Observed band size: 8-200 kDa, 36 kDa

Exposure time: 92s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.