abcam

Product datasheet

Anti-HUWE1/Mule antibody [EPR24361-13] - BSA and Azide free ab282738

Recombinant RabMAb

12 Images

Overview

Product name	Anti-HUWE1/Mule antibody [EPR24361-13] - BSA and Azide free
Description	Rabbit monoclonal [EPR24361-13] to HUWE1/Mule - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, WB, IHC-P **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, MOLT-4, 293T, NIH/3T3 and PC-12 whole cell lysate. Mouse heart tissue lysate. IHC-P: Human breast carcinoma and Mouse cardiac muscle and Rat kidney tissue. ICC/IF: HeLa, F9 and MOLT-4 cells. Flow Cyt(Intra): MOLT-4 and F9 cells.
General notes	ab282738 is the carrier-free version of **ab271032**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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.
Storage buffer	pH: 7.2 Constituent: 100% PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR24361-13
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab282738 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
Flow Cyt (Intra)		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 481 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Application notes Is unsuitable for IP.

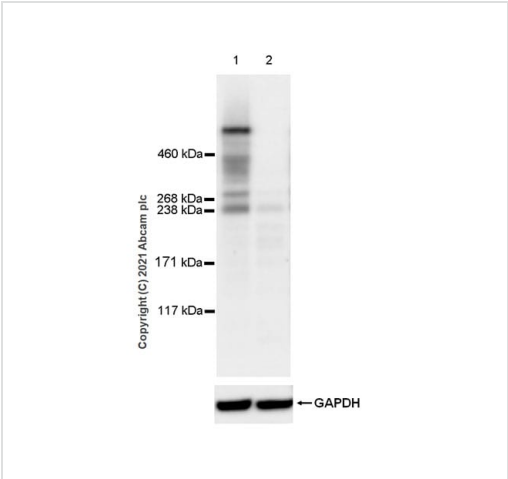
Target

Function E3 ubiquitin-protein ligase which mediates ubiquitination and subsequent proteasomal degradation of target proteins. Regulates apoptosis by catalyzing the polyubiquitination and degradation of MCL1. Mediates monoubiquitination of DNA polymerase beta (POLB) at 'Lys-41', 'Lys-61' and 'Lys-81', thereby playing a role in base-excision repair. Also ubiquitinates the p53/TP53 tumor suppressor and core histones including H1, H2A, H2B, H3 and H4. Binds to an upstream initiator-like sequence in the preprodynorphin gene. Regulates neural differentiation and proliferation by catalyzing the polyubiquitination and degradation of MYCN. May regulate abundance of CDC6 after DNA damage by polyubiquitinating and targeting CDC6 to degradation.

Tissue specificity Weakly expressed in heart, brain and placenta but not in other tissues. Expressed in a number of

	cell lines, predominantly in those from colorectal carcinomas.
Pathway	Protein modification; protein ubiquitination.
Involvement in disease	Defects in HUWE1 are the cause of mental retardation syndromic X-linked Turner type (MRXST) [MIM:300706]; also known as mental retardation and macrocephaly syndrome. MRXST shows clinical variability. Associated phenotypes include macrocephaly and variable contractures. A chromosomal microduplication involving HUWE1 and HSD17B10 is the cause of mental retardation X-linked type 17 (MRX17) [MIM:300705]; also known as mental retardation X-linked type 31 (MRX31). Mental retardation is characterized by significantly sub-average general intellectual functioning associated with impairments in adaptative behavior and manifested during the developmental period. In contrast to syndromic or specific X-linked mental retardation which also present with associated physical, neurological and/or psychiatric manifestations, intellectual deficiency is the only primary symptom of non-syndromic X-linked mental retardation.
Sequence similarities	Belongs to the TOM1/PTR1 family. Contains 1 HECT (E6AP-type E3 ubiquitin-protein ligase) domain. Contains 1 UBA domain. Contains 1 UIM (ubiquitin-interacting motif) repeat. Contains 1 WWE domain.
Domain	The HECT domain mediates inhibition of the transcriptional activity of p53.
Post-translational modifications	Phosphorylated on tyrosine; phosphorylation is probably required for its ability to inhibit TP53 transactivation. Phosphorylated upon DNA damage, probably by ATM or ATR.
Cellular localization	Cytoplasm. Nucleus. Mainly expressed in the cytoplasm of most tissues, except in the nucleus of spermatogonia, primary spermatocytes and neuronal cells (By similarity). Predominantly cytosolic or perinuclear in some colorectal carcinoma cells.

Images



Western blot - Anti-HUWE1/Mule antibody [EPR24361-13] - BSA and Azide free (ab282738)

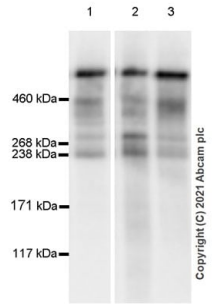
All lanes : Anti-HUWE1/Mule antibody [EPR24361-13] (**ab271032**) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : MOLT-4 (human lymphoblastic leukemia T lymphoblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 481 kDa
Observed band size: 482 kDa



Western blot - Anti-HUWE1/Mule antibody [EPR24361-13] - BSA and Azide free (ab282738)

This data was developed using **ab271032**, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
The expression pattern & observed MW are consistent with what has been described in the literature (PMID: 25883150, 28938460).

Low expression: MOLT-4 (PMID: 15567145).

Exposure time: 5.5 seconds

All lanes : Anti-HUWE1/Mule antibody [EPR24361-13] (**ab271032**) at 1/1000 dilution

Lane 1 : 293T (human embryonic kidney epithelial cell) whole cell lysate
Lane 2 : NIH/3T3 (mouse embryonic fibroblast) whole cell lysate
Lane 3 : PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

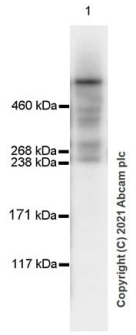
Predicted band size: 481 kDa
Observed band size: 482 kDa

This data was developed using **ab271032**, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBSTLysates were made freshly and used in WB test immediately to minimize protein degradation.

The expression pattern & observed MW are consistent with what has been described in the literature (PMID: 28938460).

Exposure time: Lane1: 3.25 seconds. Lane2-3: 5.5 seconds



Western blot - Anti-HUWE1/Mule antibody [EPR24361-13] - BSA and Azide free (ab282738)

Anti-HUWE1/Mule antibody [EPR24361-13] (**ab271032**) at 1/1000 dilution + Mouse heart tissue lysate at 20 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 481 kDa

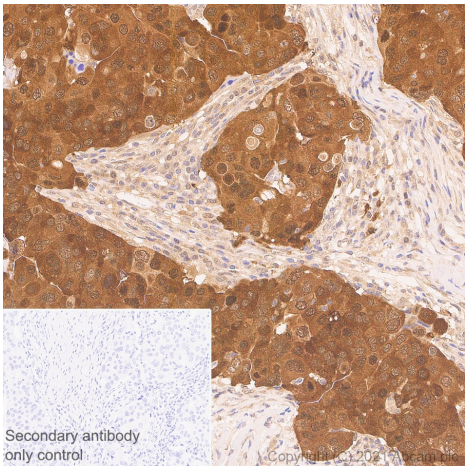
Observed band size: 482 kDa

This data was developed using **ab271032**, the same antibody clone in a different buffer formulation.

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

The expression pattern & observed MW are consistent with what has been described in the literature (PMID: 28938460).

Exposure time: 10 seconds



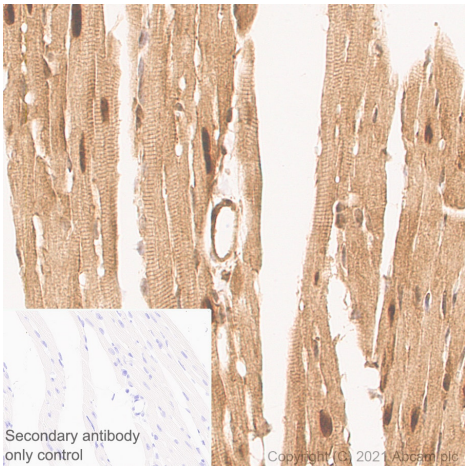
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HUWE1/Mule antibody [EPR24361-13] - BSA and Azide free (ab282738)

This data was developed using **ab271032**, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labelling HUWE1/Mule with **ab271032** at 1/1000 (0.525 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used. Positive staining in human breast carcinoma (PMID: 29375730). The section was incubated with **ab271032** for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



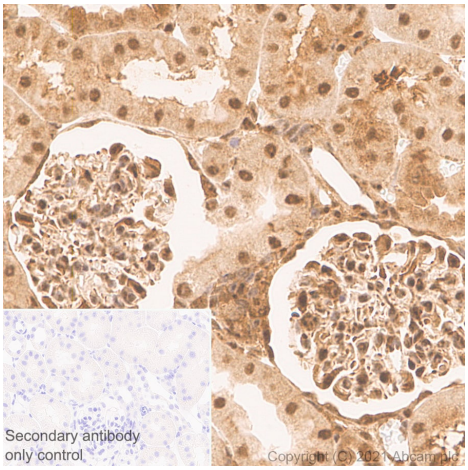
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HUWE1/Mule antibody [EPR24361-13] - BSA and Azide free (ab282738)

This data was developed using **ab271032**, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Mouse cardiac muscle tissue labelling HUWE1/Mule with **ab271032** at 1/1000 (0.525 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used. Positive staining in mouse cardiac muscle. The section was incubated with **ab271032** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



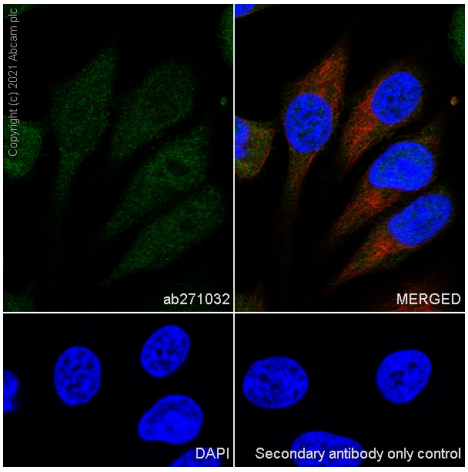
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-HUWE1/Mule antibody [EPR24361-13] - BSA and Azide free (ab282738)

This data was developed using **ab271032**, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded Rat kidney tissue labelling HUWE1/Mule with **ab271032** at 1/1000 (0.525 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used. Positive staining in rat kidney. The section was incubated with **ab271032** for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

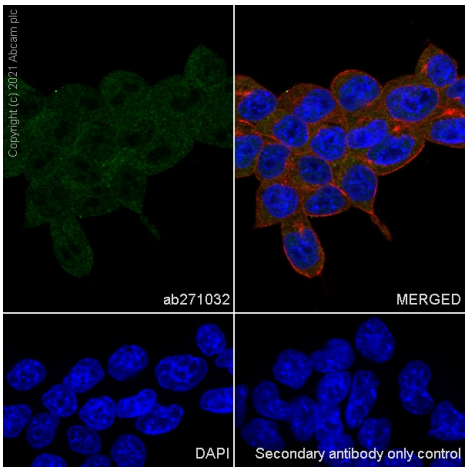


Immunocytochemistry/ Immunofluorescence - Anti-HUWE1/Mule antibody [EPR24361-13] - BSA and Azide free (ab282738)

This data was developed using **ab271032**, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa cells labelling HUWE1/Mule with **ab271032** at 1/50 (10.5 ug/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/ml) dilution (Green) Confocal image showing cytoplasmic and nuclear staining in HeLa cells is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5 ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.

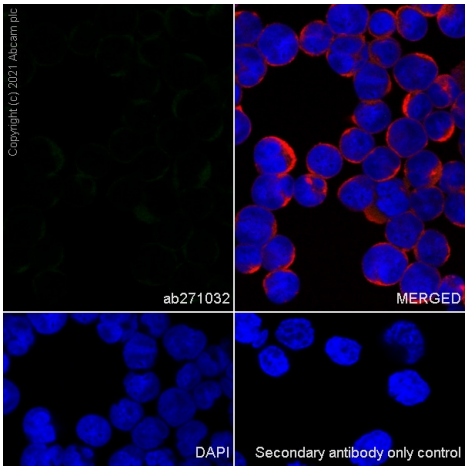


Immunocytochemistry/ Immunofluorescence - Anti-HUWE1/Mule antibody [EPR24361-13] - BSA and Azide free (ab282738)

This data was developed using **ab271032**, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized F9 cells labelling HUWE1/Mule with **ab271032** at 1/50 (10.5 ug/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 ug/ml) dilution (Green). Confocal image showing cytoplasmic and nuclear staining in F9 cells is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5 ug/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 (2 ug/ml) dilution.

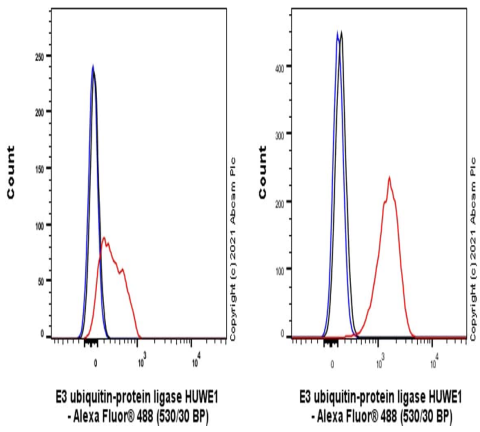


Immunocytochemistry/ Immunofluorescence - Anti-HUWE1/Mule antibody [EPR24361-13] - BSA and Azide free (ab282738)

This data was developed using **ab271032**, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized MOLT-4 cells labelling HUWE1/Mule with **ab271032** at 1/50 (10.5 ug/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2ug/ml dilution (Green). Confocal image showing weak staining in MOLT-4 cells.Low expression: MOLT-4 (PMID: 15567145) is observed. **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Red). The Nuclear counterstain was DAPI (Blue).

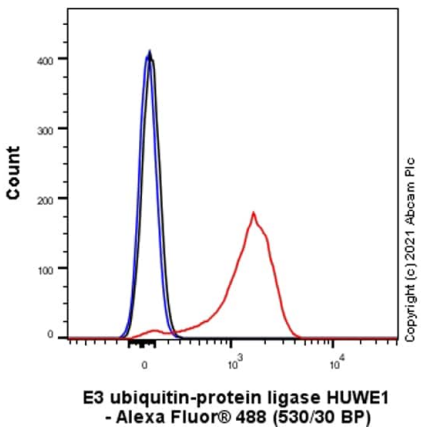
Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2ug/ml dilution.



Flow Cytometry (Intracellular) - Anti-HUWE1/Mule antibody [EPR24361-13] - BSA and Azide free (ab282738)

This data was developed using **ab271032**, the same antibody clone in a different buffer formulation.

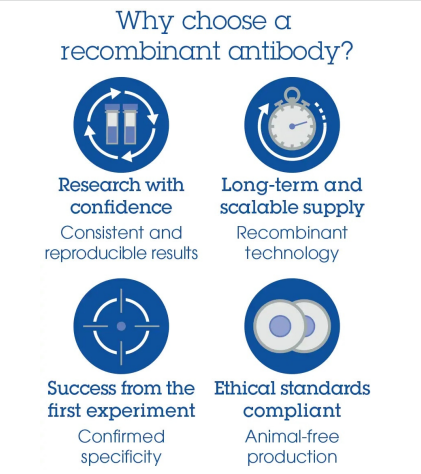
Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized MOLT-4 (human lymphoblastic leukemia T lymphoblast, Left) and HeLa (Human cervix adenocarcinoma epithelial cell, Right) cells labelling HUWE1/Mule with **ab271032** at 1/50 dilution (1ug) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody. Low expression: MOLT-4 (PMID: 15567145).



Flow Cytometry (Intracellular) - Anti-HUWE1/Mule antibody [EPR24361-13] - BSA and Azide free (ab282738)

This data was developed using **ab271032**, the same antibody clone in a different buffer formulation.

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized F9 (Mouse embryonal carcinoma epithelial cell) cells labelling HUWE1/Mule with **ab271032** at 1/50 dilution (1ug) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Anti-HUWE1/Mule antibody [EPR24361-13] - BSA and Azide free (ab282738)

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