

Product datasheet

Anti-MAGE3 antibody [EPR19065] ab223162

Recombinant RabMAb

[1 References](#) [13 Images](#)

Overview

Product name	Anti-MAGE3 antibody [EPR19065]
Description	Rabbit monoclonal [EPR19065] to MAGE3
Host species	Rabbit
Specificity	This antibody cross reacts with MAGEA2, MAGEA6, MAGEA8 and MAGEA12 and does not cross react with MAGEA1 and MAGEA4.
Tested applications	Suitable for: WB, IP, ICC/IF, IHC-P
Species reactivity	Reacts with: Human
Immunogen	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: His- and vinc-tagged human MAGEA2 recombinant protein; His-tagged human MAGEA6 MAGEA8 and MAGEA12 recombinant proteins; A-375, RPMI-6226 and HeLa whole cell lysates. IHC-P: Human testis, colon cancer, glioma, melanoma and gastric cancer tissues. ICC/IF: A-375 and HeLa cells. IP: A-375 whole cell lysate.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

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: 7.2 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol, PBS
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR19065
Isotype	IgG

Applications

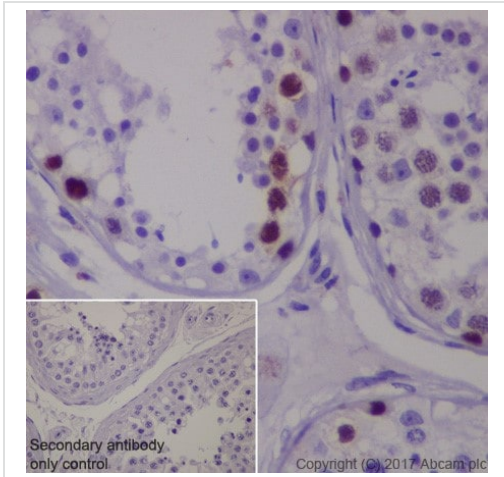
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab223162 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 45-50 kDa (predicted molecular weight: 35 kDa).
IP		1/30.
ICC/IF		1/100.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target

Function	Proposed to enhance ubiquitin ligase activity of RING-type zinc finger-containing E3 ubiquitin-protein ligases. May enhance ubiquitin ligase activity of TRIM28 and stimulate p53/TP53 ubiquitination by TRIM28. Proposed to act through recruitment and/or stabilization of the Ubl-conjugating enzyme (E2) at the E3:substrate complex. May play a role in embryonal development and tumor transformation or aspects of tumor progression. In vitro promotes cell viability in melanoma cell lines. Antigen recognized on a melanoma by autologous cytolytic T-lymphocytes.
Tissue specificity	Expressed in many tumors of several types, such as melanoma, head and neck squamous cell carcinoma, lung carcinoma and breast carcinoma, but not in normal tissues except for testes and placenta. Never expressed in kidney tumors, Leukemias and lymphomas.
Sequence similarities	Contains 1 MAGE domain.

Images

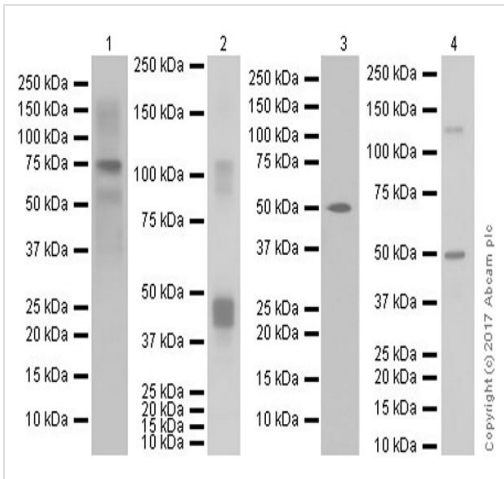


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MAGE3 antibody [EPR19065] (ab223162)

Immunohistochemical analysis of paraffin-embedded human testis tissue labeling MAGE3 with ab223162 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear staining on germ cells of human testis (PMID: 23390371). 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.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-MAGE3 antibody [EPR19065] (ab223162)

Lane 1 : Anti-MAGE3 antibody [EPR19065] (ab223162) at 1/20000 dilution

Lanes 2 & 4 : Anti-MAGE3 antibody [EPR19065] (ab223162) at 1/10000 dilution

Lane 3 : Anti-MAGE3 antibody [EPR19065] (ab223162) at 1/1000 dilution

Lane 1 : His- and vinc-tagged human MAGEA2 recombinant protein (aa1-314)

Lane 2 : His-tagged human MAGEA6 recombinant protein (aa1-314)

Lane 3 : His-tagged human MAGEA8 recombinant protein (aa1-318)

Lane 4 : His-tagged human MAGEA12 recombinant protein (aa1-314)

Lysates/proteins at 0.01 µg per lane.

Secondary

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

Developed using the ECL technique.

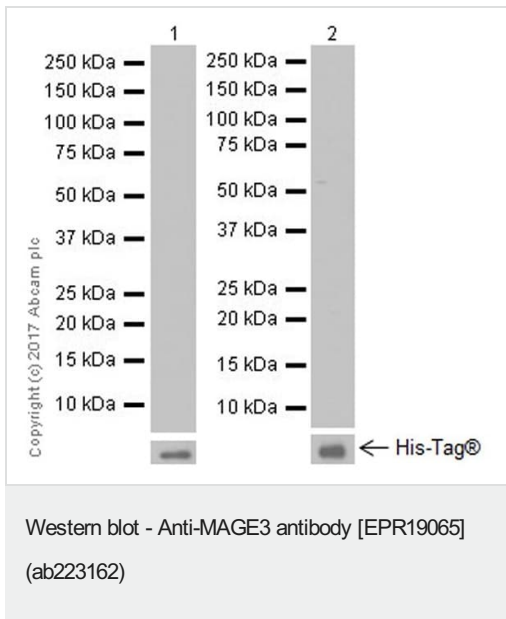
Predicted band size: 35 kDa

Observed band size: 38,41,70 kDa

Exposure time: Lane 1: 3 seconds; Lanes 2, 4: 1 second; Lane 3: 30 seconds.

Blocking/Dilution buffer: 5% NFDm/TBST.

Vinc-tag is derived from protein vinculin (P18206) and contains aa1-258 (29 kDa).



All lanes : Anti-MAGE3 antibody [EPR19065] (ab223162) at 1/1000 dilution

Lane 1 : His-tagged human MAGEA1 recombinant protein (aa1-309)

Lane 2 : His-tagged human MAGEA4 recombinant protein (aa1-317)

Lysates/proteins at 0.01 µg per lane.

Secondary

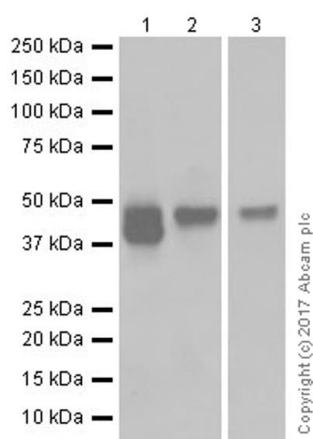
All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 35 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDm/TBST.



Western blot - Anti-MAGE3 antibody [EPR19065]
(ab223162)

All lanes : Anti-MAGE3 antibody [EPR19065] (ab223162) at 1/5000 dilution

Lane 1 : A-375 (human malignant melanoma cell line) whole cell lysate

Lane 2 : RPMI-8226 (human plasmacytoma, myeloma B lymphocyte cell line) whole cell lysate

Lane 3 : HeLa (human epithelial cell line from cervix adenocarcinoma) 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

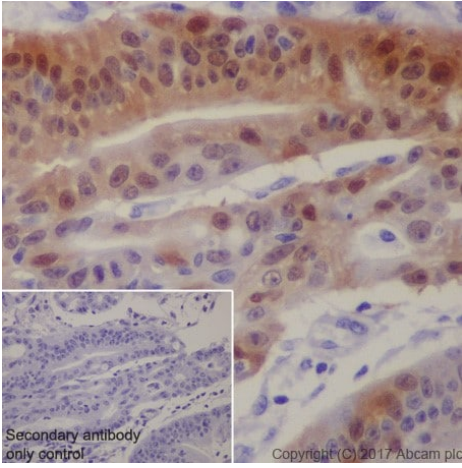
Developed using the ECL technique.

Predicted band size: 35 kDa

Exposure time: Lanes 1,2: 5 seconds; Lane 3: 2 seconds.

Blocking/Dilution buffer: 5% NFDm/TBST.

The observed molecular weight is consistent with what has been described in the literature (PMID:7757970).

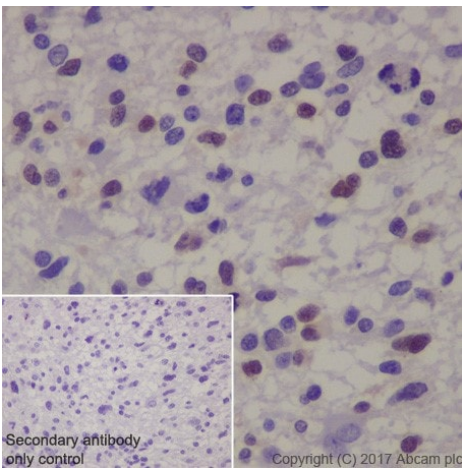


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MAGE3 antibody [EPR19065] (ab223162)

Immunohistochemical analysis of paraffin-embedded human colon cancer tissue labeling MAGE3 with ab223162 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear and cytoplasmic staining on human colon cancer (PMID: 23390371). 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.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

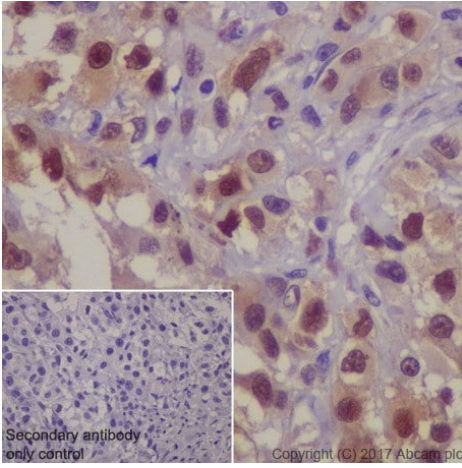


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MAGE3 antibody [EPR19065] (ab223162)

Immunohistochemical analysis of paraffin-embedded human glioma tissue labeling MAGE3 with ab223162 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear staining on human glioma (PMID: 23946777). 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.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

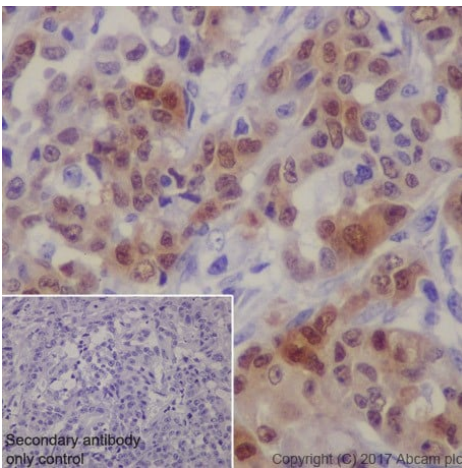


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MAGE3 antibody [EPR19065] (ab223162)

Immunohistochemical analysis of paraffin-embedded human melanoma tissue labeling MAGE3 with ab223162 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear and cytoplasmic staining on human melanoma (PMID: 26100270). 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.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

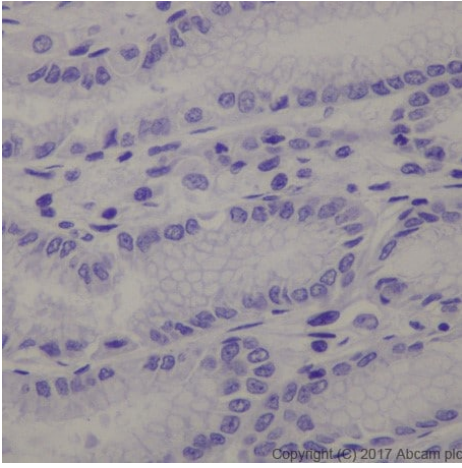


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MAGE3 antibody [EPR19065] (ab223162)

Immunohistochemical analysis of paraffin-embedded human gastric cancer tissue labeling MAGE3 with ab223162 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear and cytoplasmic staining on human gastric cancer (PMID: 16158951). 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.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



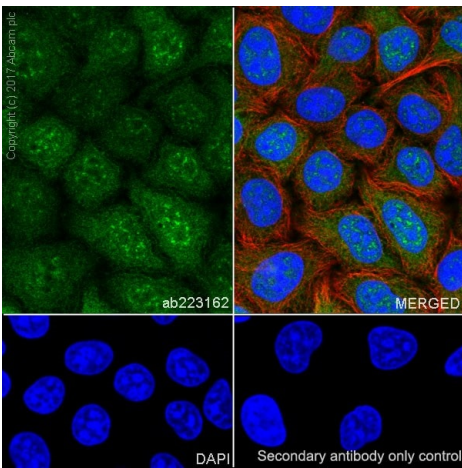
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MAGE3 antibody [EPR19065] (ab223162)

Immunohistochemical analysis of paraffin-embedded human stomach tissue labeling MAGE3 with ab223162 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Negative control: No staining on human stomach (PMID: 16158951).

Counter stained with Hematoxylin.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

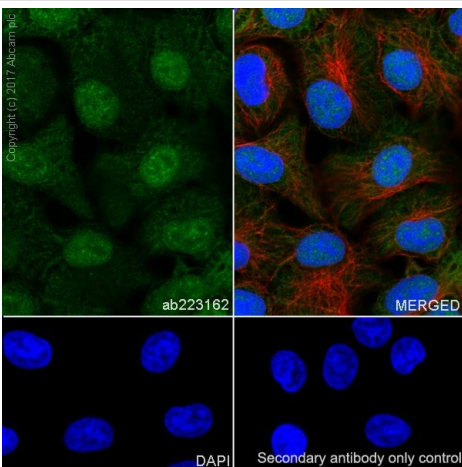


Immunocytochemistry/ Immunofluorescence - Anti-MAGE3 antibody [EPR19065] (ab223162)

Immunofluorescent analysis of 100% methanol-fixed HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling MAGE3 with ab223162 at 1/100 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 cytoplasmic staining on HeLa cell line.

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

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.

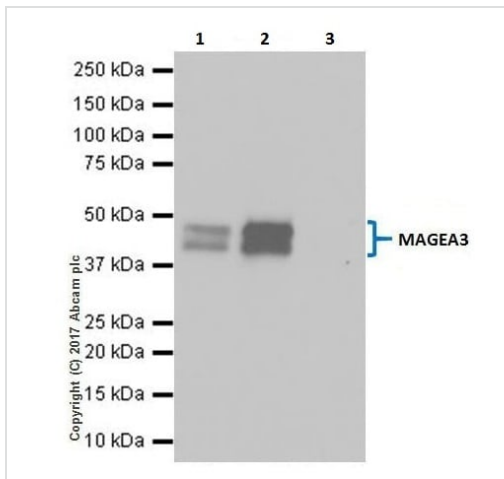


Immunocytochemistry/ Immunofluorescence - Anti-MAGE3 antibody [EPR19065] (ab223162)

Immunofluorescent analysis of 100% methanol-fixed A-375 (human malignant melanoma cell line) cells labeling MAGE3 with ab223162 at 1/100 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 cytoplasmic staining on A-375 cell line.

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

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.



Immunoprecipitation - Anti-MAGE3 antibody
[EPR19065] (ab223162)

MAGE3 was immunoprecipitated from 0.35 mg of A-375 (human malignant melanoma cell line) whole cell lysate with ab223162 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab223162 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) ([ab131366](#)), was used for detection at 1/10000 dilution.

Lane 1: A-375 whole cell lysate 10 µg (Input).

Lane 2: ab223162 IP in A-375 whole cell lysate.

Lane 3: Rabbit monoclonal IgG ([ab172730](#)) instead of ab223162 in A-375 whole cell lysate

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

Exposure time: 10 seconds.

Why choose a recombinant antibody?

 Research with confidence Consistent and reproducible results	 Long-term and scalable supply Recombinant technology
 Success from the first experiment Confirmed specificity	 Ethical standards compliant Animal-free production

Anti-MAGE3 antibody [EPR19065] (ab223162)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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