

Product datasheet

Anti-MAGE3 antibody [EPR20658] ab213722

Recombinant RabMAb

6 Images

Overview

Product name	Anti-MAGE3 antibody [EPR20658]
Description	Rabbit monoclonal [EPR20658] to MAGE3
Host species	Rabbit
Specificity	This antibody shows cross reactivity to MAGEA2 and MAGEA6 as well as weak cross reactivity to MAGEA1 and MAGEA12. It does not cross react with MAGEA4 and MAGEA8.
Tested applications	Suitable for: ICC/IF, IP, WB
Species reactivity	Reacts with: Human
Immunogen	Recombinant fragment within Human MAGE3 aa 50 to the C-terminus. The exact sequence is proprietary. Database link: P43357
Positive control	WB: RPMI-8226 and A-375 whole cell lysates; Human MAGE3, MAGEA6 and MAGEA2 recombinant proteins. ICC/IF: HeLa and A-375 cells. IP: A-375 whole cell lysate.
General notes	This product was previously labelled as MAGEA3

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 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: PBS, 0.05% BSA, 40% Glycerol

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20658
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab213722** in the following tested applications.

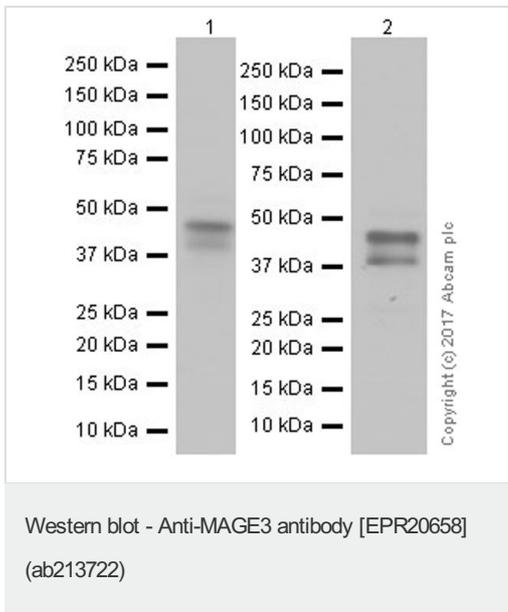
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		1/100.
IP		1/30.
WB		1/1000. Detects a band of approximately 45-50 kDa (predicted molecular weight: 34 kDa).

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



All lanes : Anti-MAGE3 antibody [EPR20658] (ab213722) at 1/1000 dilution

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

Lane 2 : RPMI-8226 (human plasmacytoma, myeloma B lymphocyte), 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: 34 kDa

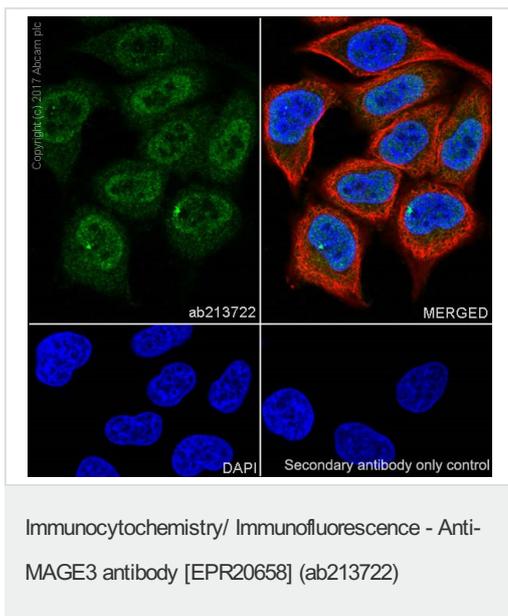
Observed band size: 45-50 kDa

[why is the actual band size different from the predicted?](#)

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDm/TBST.

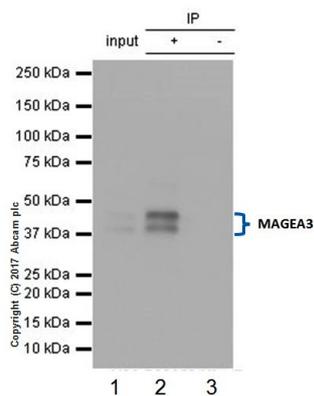
The molecular mass is consistent with what has been described in the literature (PMID: 17942928).



Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling MAGE3 with ab213722 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Nuclear and cytoplasmic staining in HeLa cell line (PMID: 23946777, PMID: 23390371) is observed.

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.



Immunoprecipitation - Anti-MAGE3 antibody [EPR20658] (ab213722)

MAGE3 was immunoprecipitated from 0.35 mg of A-375 (human malignant melanoma cell line) whole cell lysate with ab213722 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab213722 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10,000 dilution

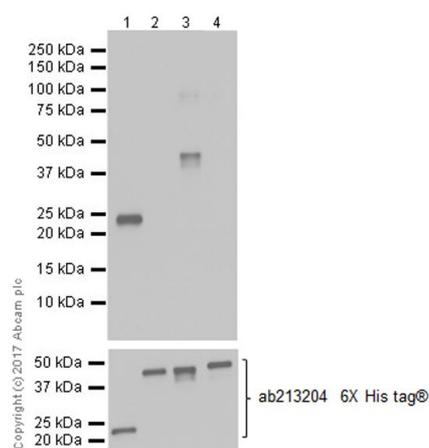
Lane 1: A-375 whole cell lysate 10 µg (Input).

Lane 2: ab213722 IP in A-375 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab213722 in A-375 whole cell lysate.

Exposure time: 1 second.

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



Western blot - Anti-MAGE3 antibody [EPR20658] (ab213722)

All lanes : Anti-MAGE3 antibody [EPR20658] (ab213722) at 1/1000 dilution

Lane 1 : His-tagged human MAGE3 recombinant protein (aa104-314)

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

Lane 3 : His-tagged Human MAGEA6 recombinant protein (aa1-314)

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

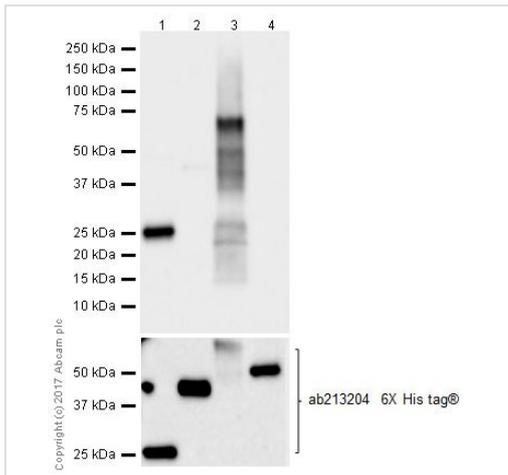
Developed using the ECL technique.

Predicted band size: 34 kDa

Observed band size: 25,39 kDa [why is the actual band size different from the predicted?](#)

Exposure time: 1 second

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-MAGE3 antibody [EPR20658]
(ab213722)

All lanes : Anti-MAGE3 antibody [EPR20658] (ab213722) at 1/1000 dilution

Lane 1 : His-tagged human MAGE3 recombinant protein (aa104-314)

Lane 2 : His-tagged human MAGEA1 recombinant protein (aa1-309)

Lane 3 : His- and vinc-tagged Human MAGEA2 recombinant protein (aa1-314)

Lane 4 : His-tagged Human MAGEA8 recombinant protein (aa1-318)

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.

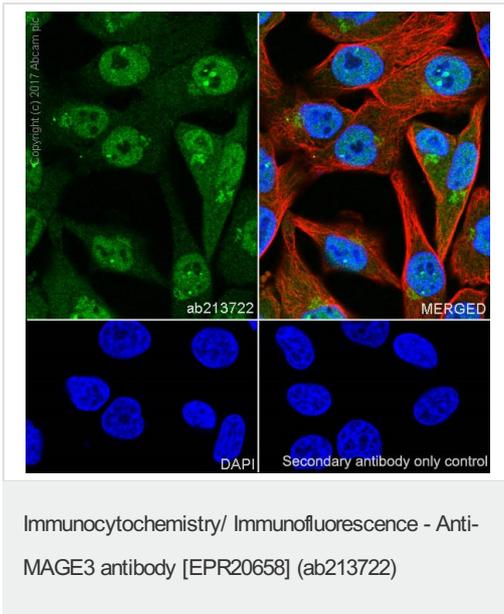
Predicted band size: 34 kDa

Observed band size: 25,39,75 kDa [why is the actual band size different from the predicted?](#)

Exposure time: 7 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The blot was developed on a BIO-RAD® ChemiDoc™ MP instrument.



Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A-375 (human malignant melanoma cell line) cells labeling MAGE3 with ab213722 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Nuclear and cytoplasmic staining in A-375 cell line (PMID: 23946777, PMID: 23390371) is observed.

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