

## Product datasheet

# Anti-LINE-1 ORF1p antibody [EPR22227-54] ab230966

Recombinant **RabMAb**

9 Images

### Overview

<b>Product name</b>	Anti-LINE-1 ORF1p antibody [EPR22227-54]
<b>Description</b>	Rabbit monoclonal [EPR22227-54] to LINE-1 ORF1p
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> WB, IHC-P, ICC/IF, Flow Cyt, IP
<b>Species reactivity</b>	<b>Reacts with:</b> Human
<b>Immunogen</b>	Recombinant fragment within Human LINE-1 ORF1p aa 150 to the C-terminus. The exact sequence is proprietary. Database link: <a href="#">Q9UN81</a>
<b>Positive control</b>	WB: K562, MCF7 and NCCIT whole cell lysates. IP: NCCIT whole cell lysate. IHC-P: Human lung cancer, pancreatic cancer and colon cancer tissue. ICC/IF: NCCIT and MCF7 cells. Flow cyt: NCCIT cells.
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p> <p>Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough.</p> <p>Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.</p> <p>We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications &amp; species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise<sup>™</sup> guarantee.</p> <p>In preparation for this, we have started to update the applications &amp; species that this product is Abpromise guaranteed for.</p> <p>We are also updating the applications &amp; species that this product has been "predicted to work</p>

with," however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, as well as customer reviews and Q&As.

## Properties

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<b>Form</b>	Liquid
<b>Storage instructions</b>	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.
<b>Storage buffer</b>	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR22227-54
<b>Isotype</b>	IgG

## Applications

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Our [Abpromise guarantee](#) covers the use of **ab230966** 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 33, 42 kDa (predicted molecular weight: 40 kDa).
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/100.
Flow Cyt		1/500.
IP		1/30.

## Target

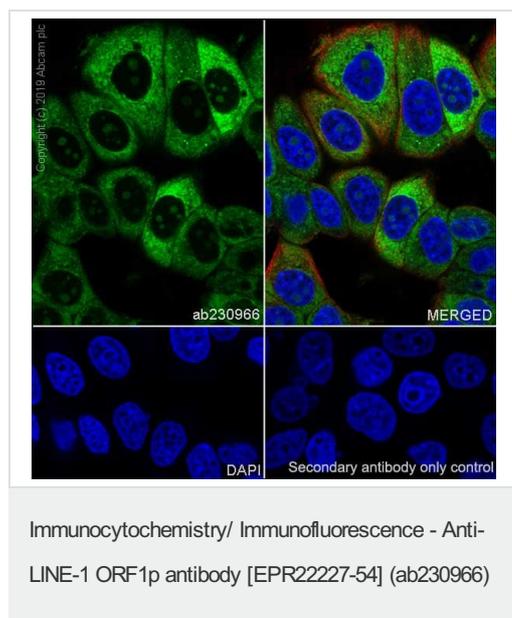
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### Relevance

Domain: The coiled coil domain mediates homotrimerization. The RRM and the CTD domain are both required for proper RNA-binding activity. Function: Nucleic acid-binding protein which is essential for retrotransposition of LINE-1 elements in the genome. Functions as a nucleic acid chaperone binding its own transcript and therefore preferentially mobilizing the transcript from which they are encoded. Long interspersed element-1/LINE-1/L1 retrotransposons are present in

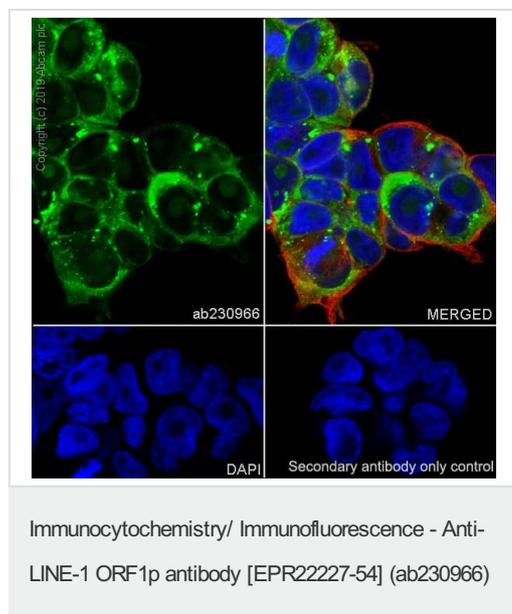
more than 500'000 full (6 kb) or truncated copies in the human genome. Most of them are inactive but 80 to 100 of those elements could be transcribed, translated and active in any individual. An active LINE-1 encodes for 2 proteins translated from a single RNA containing 2 non-overlapping ORFs, ORF1 and ORF2. ORF1p is described in this entry as a representative of all ORF1p potentially expressed by active elements. ORF2p is described in the related entry AC O00370. PTM: Polyubiquitinated, probably by UBR2, which induces its degradation. Similarity: Belongs to the transposase 22 family.

## Images



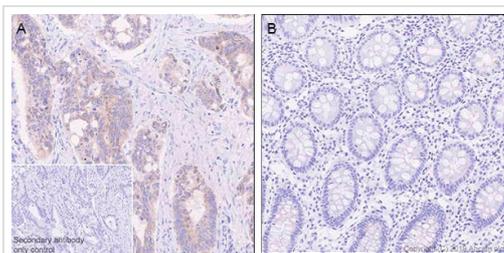
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized MCF7 (human breast adenocarcinoma cell line) cells labeling LINE-1 ORF1p with ab230966 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic with punctate foci and nucleolar staining in MCF7 cells (PMID: 15028673; 17562864). The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) (ab195889) at 1/200 dilution (red).

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



Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NCCIT (human pluripotent embryonic carcinoma cell line) cells labeling LINE-1 ORF1p with ab230966 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic with punctate foci and nucleolar staining in NCCIT cells (PMID: 15028673; 17562864). The nuclear counterstain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) (ab195889) at 1/200 dilution (red).

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



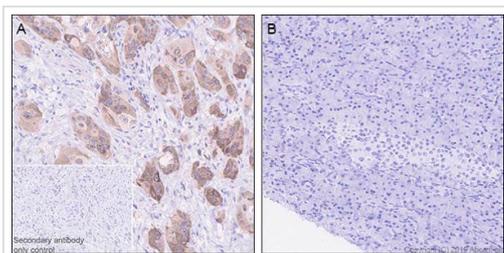
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LINE-1 ORF1p antibody [EPR22227-54] (ab230966)

Immunohistochemical analysis of paraffin-embedded human colon cancer tissue (Panel A) and adjacent human normal colon tissue (Panel B) labeling LINE-1 ORF1p with ab230966 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Cytoplasmic staining in colon cancer (Panel A) but negative in adjacent normal tissue (Panel B). (PMID: 24607009) is observed. Counterstained 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.

Heat mediated antigen retrieval using [ab93684](#) (Tris/EDTA buffer, pH 9.0).

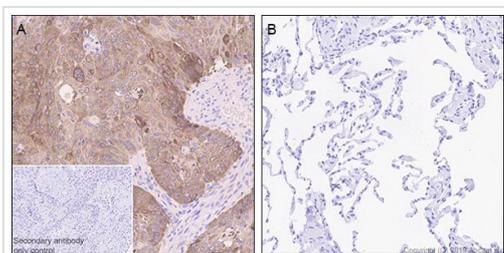


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LINE-1 ORF1p antibody [EPR22227-54] (ab230966)

Immunohistochemical analysis of paraffin-embedded human pancreatic cancer tissue (Panel A) and adjacent human normal pancreatic tissue (Panel B) labeling LINE-1 ORF1p with ab230966 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Cytoplasmic staining in pancreatic cancer (Panel A) but negative in adjacent normal tissue (Panel B). (PMID: 24607009) is observed. Counterstained 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.

Heat mediated antigen retrieval using [ab93684](#) (Tris/EDTA buffer, pH 9.0).



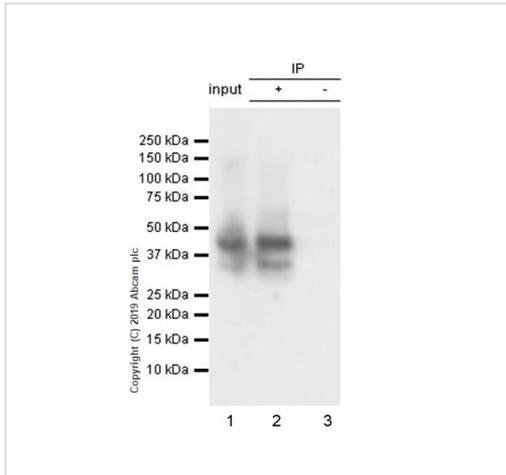
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LINE-1 ORF1p antibody [EPR22227-54] (ab230966)

Immunohistochemical analysis of paraffin-embedded human lung cancer tissue (Panel A) and adjacent human normal lung tissue (Panel B) labeling LINE-1 ORF1p with ab230966 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Cytoplasmic staining in lung cancer (Panel A) but negative in adjacent normal tissue (Panel B) (PMID: 24607009) is observed. Counterstained 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.

Heat mediated antigen retrieval using [ab93684](#) (Tris/EDTA buffer, pH 9.0).



Immunoprecipitation - Anti-LINE-1 ORF1p antibody  
[EPR22227-54] (ab230966)

LINE-1 ORF1p was immunoprecipitated from 0.35 mg of NCCIT (human pluripotent embryonic carcinoma cell line) whole cell lysate with ab230966 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab230966 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/5000 dilution.

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

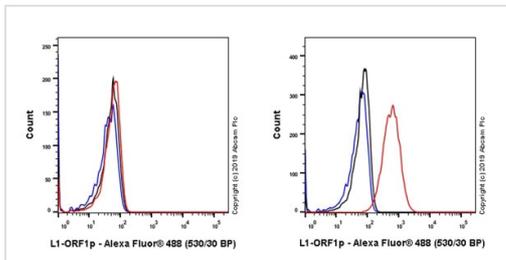
**Lane 2:** ab230966 IP in NCCIT whole cell lysate.

**Lane 3:** Rabbit monoclonal IgG (ab172730) instead of ab230966 in NCCIT whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFD/MTBST.

Exposure time: 1 second.

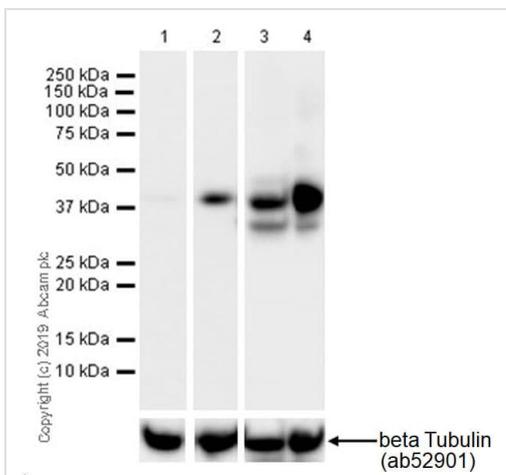
The lower 33 kDa band is likely to be a truncated form (PMID: 29505568).



Flow Cytometry - Anti-LINE-1 ORF1p antibody  
[EPR22227-54] (ab230966)

Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HCT 116 (human colorectal carcinoma cell line) (Left panel) and NCCIT (human pluripotent embryonic carcinoma cell line) (Right panel) cells labeling LINE-1 ORF1p with ab230966 at 1/500 dilution (red) compared with 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) (ab150077), at 1/2000 dilution was used as the secondary antibody.

**Negative control:** The expression level on HCT 116 (low) and NCCIT (high) is consistent with literature (PMID: 27016617).



Western blot - Anti-LINE-1 ORF1p antibody  
[EPR22227-54] (ab230966)

**All lanes :** Anti-LINE-1 ORF1p antibody [EPR22227-54] (ab230966) at 1/1000 dilution

**Lane 1 :** HCT 116 (human colorectal carcinoma cell line) whole cell lysate

**Lane 2 :** K562 (human chronic myelogenous leukemia cell line from bone marrow ) whole cell lysate

**Lane 3 :** MCF7 (human breast adenocarcinoma cell line) whole cell lysate

**Lane 4 :** NCCIT (human pluripotent embryonic carcinoma cell line) 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

**Predicted band size:** 40 kDa

**Observed band size:** 33,42 kDa [why is the actual band size different from the predicted?](#)

**Exposure time:** 1 second

Blocking and dilution buffer: 5% NFDm/TBST.

Exposure times.

Lanes 1 and 2: 37 seconds.

Lanes 3 and 4: 15 seconds.

The molecular weight observed is consistent with what has been described in the literature (PMID: 29505568).

The lower 33 kDa band is likely to be a truncated form (PMID: 29505568).

**Control cell line:** HCT 116 (low-expression, PMID: 27016617).

Why choose a recombinant antibody?

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

Anti-LINE-1 ORF1p antibody [EPR22227-54]  
(ab230966)

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

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