Product datasheet

Anti-Ret antibody [EPR2871] ab134100

Overview

Product name
Anti-Ret antibody [EPR2871]

Description
Rabbit monoclonal [EPR2871] to Ret

Host species
Rabbit

Tested applications
Suitable for: ELISA, WB, IP, IHC-P, ICC/IF

Unsuitable for: Flow Cyt

Species reactivity
Reacts with: Mouse, Rat, Human

Immunogen
Synthetic peptide within Human Ret aa 1100 to the C-terminus (C terminal). The exact sequence is proprietary.

Database link: P07949
(Peptide available as ab219199)

Positive control
WB: Neuro-2a, SH-SY5Y and TT cell lysates and mouse and rat brain tissue lysates. IHC-P: Human thyroid gland carcinoma, gastric carcinoma and colon tissues; Mouse colon tissue; Human medullary thyroid carcinoma (MTC) samples from GTCAC haplotype carriers & non-carriers. ICC/IF: Neuro-2a and TT cells. IP: Neuro-2a cell lysate.

General notes
Our RabMab® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

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. Stable for 12 months at -20°C.

Storage buffer
pH: 7.40
Preservative: 0.01% Sodium azide
Constituents: 40% Glycerol, 0.05% BSA, 59% PBS

Purity  Protein A purified
Clonality  Monoclonal
Clone number  EPR2871
Isotype  IgG

Applications

Our Abpromise guarantee covers the use of ab134100 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>ELISA</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 0.5 µg/ml.</td>
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<tr>
<td>IP</td>
<td></td>
<td>1/10 - 1/100.</td>
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<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/50 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.</td>
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<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/50 - 1/100.</td>
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Application notes  Is unsuitable for Flow Cyt.

Target

Function  Probable receptor with tyrosine-protein kinase activity; important for development.

Involvement in disease  Defects in RET may be a cause of colorectal cancer (CRC) [MIM:114500].
Defects in RET are a cause of Hirschsprung disease (HSCR) [MIM:142623]. HSCR is a genetic disorder of neural crest development characterized by the absence of intramural ganglion cells in the hindgut, often resulting in intestinal obstruction. Occasionally, MEN2A or FMTC occur in association with HSCR.
Defects in RET are the cause of medullary thyroid carcinoma (MTC) [MIM:155240]. MTC is a rare tumor derived from the C cells of the thyroid. Three hereditary forms are known, that are transmitted in an autosomal dominant fashion: (a) multiple neoplasia type 2A (MEN2A), (b) multiple neoplasia type 2B (MEN2B) and (c) familial MTC (FMTC), which occurs in 25-30% of MTC cases and where MTC is the only clinical manifestation.
Defects in RET are the cause of multiple neoplasia type 2B (MEN2B) [MIM:162300]. MEN2B is an uncommon inherited cancer syndrome characterized by predisposition to MTC and pheochromocytoma which is associated with marfanoid habitus, mucosal neuromas, skeletal and ophthalmic abnormalities, and ganglioneuromas of the intestine tract. Then the disease progresses rapidly with the development of metastatic MTC and a pheochromocytoma in 50% of cases.
Defects in RET are a cause of susceptibility to pheochromocytoma (PCC) [MIM:171300]. A catecholamine-producing tumor of chromaffin tissue of the adrenal medulla or sympathetic paraganglia. The cardinal symptom, reflecting the increased secretion of epinephrine and norepinephrine, is hypertension, which may be persistent or intermittent.
Defects in RET are the cause of multiple neoplasia type 2A (MEN2A) [MIM:171400]; also known as multiple neoplasia type 2 (MEN2). MEN2A is the most frequent form of medullary thyroid cancer (MTC). It is an inherited cancer syndrome characterized by MTC, phaeochromocytoma and/or hyperparathyroidism.

Defects in RET are a cause of thyroid papillary carcinoma (TPC) [MIM:188550]. TPC is a common tumor of the thyroid that typically arises as an irregular, solid or cystic mass from otherwise normal thyroid tissue. Papillary carcinomas are malignant neoplasm characterized by the formation of numerous, irregular, finger-like projections of fibrous stroma that is covered with a surface layer of neoplastic epithelial cells. Note=Chromosomal aberrations involving RET are found in thyroid papillary carcinomas. Inversion inv(10)(q11.2;q21) generates the RET/CCDC6 (PTC1) oncogene; inversion inv(10)(q11.2;q11.2) generates the RET/NCOA4 (PTC3) oncogene; translocation t(10;14)(q11;q32) with GOLGA5 generates the RET/GOLGA5 (PTC5) oncogene; translocation t(8;10)(p21.3;q11.2) with PCM1 generates the PCM1/RET fusion; translocation t(6;10)(p21.3;q11.2) with RFP generates the Delta RFP/RET oncogene; translocation t(1;10) (p13;q11) with TRIM33 generates the TRIM33/RET (PTC7) oncogene; translocation t(7;10) (q32;q11) with TRIM24/TIF1 generates the TRIM24/RET (PTC6) oncogene. The PTC5 oncogene has been found in 2 cases of PACT in children exposed to radioactive fallout after Chernobyl. A chromosomal aberration involving TRIM27/RFP is found in thyroid papillary carcinomas. Translocation t(6;10)(p21.3;q11.2) with RET. The translocation generates TRIM27/RET and delta TRIM27/RET oncogenes.

Defects in RET are a cause of renal adysplasia (RADYS) [MIM:191830]; also known as renal agenesis or renal aplasia. Renal agenesis refers to the absence of one (unilateral) or both (bilateral) kidneys at birth. Bilateral renal agenesis belongs to a group of perinatally lethal renal diseases, including severe bilateral renal dysplasia, unilateral renal agenesis with contralateral dysplasia and severe obstructive uropathy.

Defects in RET are a cause of congenital central hypoventilation syndrome (CCHS) [MIM:209880]; also known as congenital failure of autonomic control or Ondine curse. CCHS is a rare disorder characterized by abnormal control of respiration in the absence of neuromuscular or lung disease, or an identifiable brain stem lesion. A deficiency in autonomic control of respiration results in inadequate or negligible ventilatory and arousal responses to hypercapnia and hypoxemia.

Sequence similarities
Belongs to the protein kinase superfamily. Tyr protein kinase family.
Contains 1 cadherin domain.
Contains 1 protein kinase domain.

Post-translational modifications
Autophosphorylated on C-terminal tyrosine residues upon ligand stimulation. Dephosphorylated by PTPRJ on Tyr-905, Tyr-1015 and Tyr-1062.

Cellular localization
Membrane.

Images

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Immunostaining of the Ret proto-oncogene in Medullary thyroid carcinoma (MTC) samples from GTCAC haplotype carriers & non-carriers.

Two representative slices of Ret immunostaining in a sample carrier S836S/3’UTR (GTCAC haplotype) (left) and non-carrier of this haplotype (right).

Anti-Ret antibody [EPR2871] (ab134100) at 1/1000 dilution (purified) + TT (human thyroid carcinoma cell line) whole cell lysate at 10 µg

Secondary

Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 124 kDa
Observed band size: 155,175 kDa

why is the actual band size different from the predicted?

Blocking and dilution buffer: 5% NFDM/TBST

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human gastric carcinoma tissue labelling Ret with purified ab134100 at a dilution of 1/50. Heat mediated antigen retrieval was performed using EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.
Immunocytochemistry/Immunofluorescence analysis of Neuro-2a cells labelling Ret with purified ab134100 at a dilution of 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse anti-tubulin (1/1000) and ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used.

Control 1: primary antibody (1/100) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: ab7291 (1/1000) and secondary antibody, ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).

Anti-Ret antibody [EPR2871] (ab134100) at 1/5000 dilution (purified) + SH-SY5Y (human neuroblastoma cell line from bone marrow) whole cell lysate at 10 µg

Secondary
Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 124 kDa
Observed band size: 155,175 kDa why is the actual band size different from the predicted?

Blocking and dilution buffer: 5% NFDM/TBST
Formaldehyde-fixed, paraffin-embedded human colon tissue stained for Ret using ab134100 at 1/80 dilution in immunohistochemical analysis, followed by Goat anti-Rabbit IgG (HRP antibody).

Immunocytochemistry/Immunofluorescence analysis of TT (human thyroid carcinoma epithelial cell) cells labelling Ret with purified ab134100 at a dilution of 1/200. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (1/200) was used as a counter stain.

-ve control: Caco-2 (PMID: 10811228) cells stained with primary antibody (1/100) and secondary antibody, ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).

All lanes : Anti-Ret antibody [EPR2871] (ab134100) at 1/1000 dilution (purified)

Lane 1 : Mouse brain tissue
Lane 2 : Rat brain tissue

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 124 kDa
Observed band size: 155,175 kDa why is the actual band size different from the predicted?
Blocking and dilution buffer: 5% NFDM/TBST

ELISA antigen dose-response curve using purified ab134100 at 0-1000 ng/ml. Antigen concentration of 1000 ng/mL. An Alkaline-Phosphatase-conjugated goat anti-rabbit IgG (H+L) (1/2500) was used as the secondary antibody.

ELISA - Anti-Ret antibody [EPR2871] (ab134100)

All lanes : Anti-Ret antibody [EPR2871] (ab134100) at 1/10000 dilution (unpurified)

Lane 1 : Neuro-2a (mouse neuroblastoma cell line) cell lysate
Lane 2 : TT (human thyroid carcinoma cell line) cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution

Predicted band size: 124 kDa

Lane 1 : Mouse brain tissue lysate
Lane 2 : Rat brain tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

Predicted band size: 124 kDa
Observed band size: 155 kDa

why is the actual band size different from the predicted?
Exposure time: 3 minutes

Blocking and Diluting buffer and concentration: 5% NFDM/TBST

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thyroid gland carcinoma tissue labelling Ret with unpurified ab134100 at a dilution of 1/250.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse colon tissue labelling Ret with unpurified ab134100 at a dilution of 1/50 followed by HRP-conjugated goat anti-rabbit IgG (H&L) (ab97051, 1/500). Counter stained with hematoxylin.
Anti-Ret antibody [EPR2871] (ab134100) at 1/10000 dilution (purified) + Neuro-2a (mouse neuroblastoma cell line) whole cell lysate at 10 µg

**Secondary**
Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/2000 dilution

**Predicted band size:** 124 kDa

**Observed band size:** 155,175 kDa why is the actual band size different from the predicted?

**Exposure time:** 3 minutes

Blocking and dilution buffer: 5% NFDM /TBST.
We are unsure about the nature of 40kDa to 60kDa bands, they might be the intracellular fragments of Ret.

ab134100 (purified) at 1/40 immunoprecipitating Ret in Neuro-2a mMouse neuroblastoma cell line) whole cell lysate.

Lane 1 (input): Neuro-2a whole cell lysate (10µg)

Lane 2 (+): ab134100 + Neuro-2a whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab134100 in Neuro-2a whole cell lysate.

For western blotting, ab134100 was used at 1/1000 followed by a HRP-conjugated anti-rabbit IgG (specific to the non-reduced form of IgG, 1/1500).

Blocking buffer and dilution concentration: 5% NFDM/TBST.
We are unsure about the nature of 40kDa to 60kDa bands, they might be the intracellular fragments of Ret.
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