abcam

ab325656

Anti-ROR gamma antibody [EPR27137-10] - BSA and Azide free

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR27137-10
Purification technique	Affinity purification Protein A
Concentration	1.013 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Transfected cell lysate - Human, Human
Dilution info	-

Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Human, Transfected cell lysate - Human
Dilution info	-
Notes	-

Target data

See full target information RORC

Function

Nuclear receptor that binds DNA as a monomer to ROR response elements (RORE) containing a single core motif half-site 5'-AGGTCA-3' preceded by a short A-T-rich sequence. Key regulator of cellular differentiation, immunity, peripheral circadian rhythm as well as lipid, steroid, xenobiotics and glucose metabolism (PubMed:19381306, PubMed:19965867, PubMed:20203100, PubMed:22789990, PubMed:26160376). Considered to have intrinsic transcriptional activity, have some natural ligands like oxysterols that act as agonists (25-hydroxycholesterol) or inverse agonists (7-oxygenated sterols), enhancing or repressing the transcriptional activity, respectively (PubMed:19965867, PubMed:22789990). Recruits distinct combinations of cofactors to target gene regulatory regions to modulate their transcriptional expression, depending on the tissue, time and promoter contexts. Regulates the circadian expression of clock genes such as CRY1, BMAL1 and NR1D1 in peripheral tissues and in a tissue-selective manner. Competes with NR1D1 for binding to their shared DNA response element on some clock genes such as BMAL1, CRY1 and NR1D1 itself, resulting in NR1D1-mediated repression or RORC-mediated activation of the expression, leading to the circadian pattern of clock genes expression. Therefore influences the period length and stability of the clock. Involved in the regulation of the rhythmic expression of genes involved in glucose and lipid metabolism, including PLIN2 and AVPR1A (PubMed:19965867). Negative regulator of adipocyte differentiation through the regulation of early phase genes expression, such as MMP3. Controls adipogenesis as well as adipocyte size and modulates insulin sensitivity in obesity. In liver, has specific and redundant functions with RORA as positive or negative modulator of expression of genes encoding phase I and Phase II proteins involved in the metabolism of lipids, steroids and xenobiotics, such as SULT1E1. Also plays a role in the regulation of hepatocyte glucose metabolism through the regulation of G6PC1 and PCK1 (PubMed:19965867). Regulates the rhythmic expression of PROX1 and promotes its nuclear localization (PubMed:19381306, PubMed:19965867, PubMed:20203100, PubMed:22789990, PubMed:26160376). Plays an indispensable role in the induction of IFN-gamma dependent anti-mycobacterial systemic immunity (PubMed:26160376). Isoform 2. Nuclear receptor essential for thymopoiesis and the development of several secondary lymphoid tissues, including lymph nodes and Peyer's patches (PubMed:21499262). Required for the generation of LTi (lymphoid tissue inducer) cells (By similarity). Regulates thymocyte survival through DNA-binding on ROREs of target gene promoter regions and recruitment of coactivaros via the AF-2 (By similarity). Also plays a key role, downstream of IL6 and TGFB and synergistically with RORA, for lineage specification of uncommitted CD4(+) T-helper (T(H)) cells into T(H)17 cells, antagonizing the T(H)1 program (PubMed:21499262). Probably regulates IL17 and IL17F expression on T(H) by binding to the essential enhancer conserved non-coding sequence 2 (CNS2) in the IL17-IL17F locus (PubMed:21499262). Plays a key role in tolerance to gut microbiota and foreign antigens in food by mediating differentiation of peripherally-induced regulatory T-cells (pTreg), which suppress inflammatory responses to commensal microorganisms and food proteins (By similarity). Specifically expressed in antigen-presenting cell populations, such as (1) type 3 innate lymphoid cells (ILC3s), which are required for tolerance to gut microbiota, (2) Thetis cells, also named Janus cells, which are enriched in gut lymph nodes during early life and are required for oral tolerance, and (3) a subset of dendritic cells (By similarity). Following activation by an unknown ligand in

antigen-presenting cells, promotes recruitment of transcription regulators, such as PRDM16 or ZBTB46, and drives differentiation of pTreg cells, which maintain immune tolerance and prevent autoimmune diseases (By similarity). May also play a role in the pre-TCR activation cascade leading to the maturation of alpha/beta T-cells and may participate in the regulation of DNA accessibility in the TCR-J(alpha) locus (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C

Notes

ab325656 is the carrier-free version of ab325651

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

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

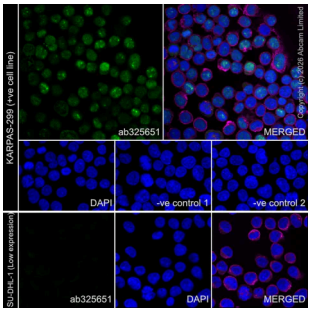
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

5 product images



Immunocytochemistry/ Immunofluorescence - Anti-ROR gamma antibody [EPR27137-10] - BSA and Azide free (ab325656)

ROR gamma Immunocytochemistry/ Immunofluorescence staining of KARPAS-299 (human T cell lymphoma cell) using rabbit Anti-ROR gamma antibody

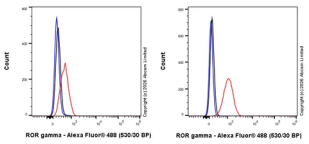
This data was developed using ab325651, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized KARPAS-299 (human T cell lymphoma cell) and SU-DHL-1 (human histiocytic lymphoblast-like cell) cells labelling ROR gamma with ab325651 at 1/200 dilution (Green).

Confocal image showing mainly nuclear staining in KARPAS-299 cells (shown in green). The counterstain was observed in magenta. Nuclear DNA was labelled with DAPI (shown in blue).

Low expression: SU-DHL-1(PMID:29588546).
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

-ve control 1: ab325651 at 1/200 dilution, followed by ab150120 at 1/1000 dilution.
-ve control 2: ab7291 at 1/1000 dilution, followed by Tyramide SuperBoost™ with Alexa Fluor™ 488 kit.



Flow Cytometry (Intracellular) - Anti-ROR gamma antibody [EPR27137-10] - BSA and Azide free (ab325656)

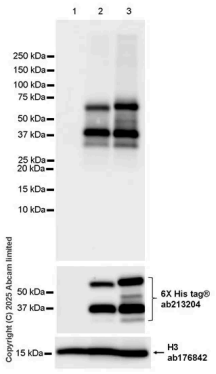
ROR gamma Flow Cytometry (Intracellular) staining of karpas-299 (human T cell lymphoma cell, Right) using rabbit Anti-ROR gamma antibody

This data was developed using ab325651, the same antibody clone in a different buffer formulation.

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized SU-DHL-1 (human histiocytic lymphoblast-like cell, Left) and karpas-299 (human T cell lymphoma cell, Right) cells labelling ROR gamma with ab325651 at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.

Low expression: SU-DHL-1(PMID: 29588546).



Western blot - Anti-ROR gamma antibody [EPR27137-10] - BSA and Azide free (ab325656)

ROR gamma Western blot staining using rabbit Anti-ROR gamma antibody

This data was developed using ab325651, the same antibody clone in a different buffer formulation.

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

The antibody reacts with both the ROR gamma and ROR gamma t isoforms.

In Western blot, Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) 1:100000 (15Kda);
Anti-6X His tag® antibody [EPR20547] - ChIP Grade (ab213204) (1:5000)(37,55kDa).

All lanes:
Western blot - Anti-ROR gamma antibody [EPR27137-10] (ab325651) at 1/1000 dilution

Lane 1:
293T (human embryonic kidney epithelial cell) cells transfected with an empty vector containing a myc-His-tag® whole cell lysate at 20 µg

Lane 2:
293T cells transfected with human ROR gamma t expression vector containing a myc-His-tag® whole cell lysate at 20 µg

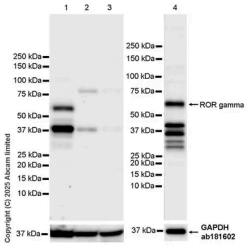
Lane 3:
293T cells transfected with human ROR gamma expression vector containing a myc-His-tag® whole cell lysate at 10 µg

Secondary

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

Observed band size: 37 kDa, 55 kDa, 15 kDa

Exposure time: 180s



Western blot - Anti-ROR gamma antibody [EPR27137-10] - BSA and Azide free (ab325656)

ROR gamma Western blot staining using rabbit Anti-ROR gamma antibody

This data was developed using ab325651, the same antibody clone in a different buffer formulation.

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

Negative control: HepG2, A549

The identity of the lower MW band at approximately 37 kDa is unknown.

Lanes 1-3 are incubated with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/20000 dilution and lane 4 are incubated with Goat Anti-Rabbit IgG (HRP) at 1/2000 dilution.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) (1:200000) (36KDa).

All lanes:
Western blot - Anti-ROR gamma antibody [EPR27137-10] (ab325651) at 1/1000 dilution

Lane 1:
SR (human pleural effusion lymphoblast) whole cell lysate at 20 µg

Lane 2:
HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 3:
A549 (human lung carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 4:
Human thymus tissue lysate at 20 µg

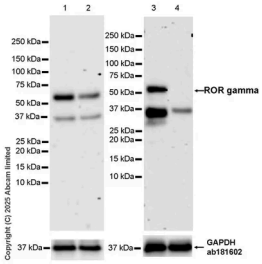
Secondary

Lanes 1 - 3:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Lane 4:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Observed band size: 55 kDa, 36 kDa

Exposure time: 59s



Western blot - Anti-ROR gamma antibody [EPR27137-10] - BSA and Azide free (ab325656)

ROR gamma Western blot staining using rabbit Anti-ROR gamma antibody

This data was developed using ab325651, the same antibody clone in a different buffer formulation.

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

Low expression: SU-DHL-1(PMID: 29588546).

The identity of the lower MW band at approximately 37 kDa is unknown.

Lanes 1-2 are incubated with Goat Anti-Rabbit IgG (HRP) at 1/2000 dilution and lanes 3-4 are incubated with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/20000 dilution.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) (1:200000) (36KDa).

All lanes:
Western blot - Anti-ROR gamma antibody [EPR27137-10] (ab325651) at 1/1000 dilution

Lane 1:
KARPAS-299(human T cell lymphoma cell) transfected with scrambled siRNA control whole cell lysate at 40 µg

Lane 2:
KARPAS-299 transfected with siRNA specifically targeting ROR gamma whole cell lysate at 40 µg

Lane 3:
KARPAS-299 (human T cell lymphoma cell) whole cell lysate at 40 µg

Lane 4:

SU-DHL-1 (human histiocytic lymphoblast-like cell) whole cell lysate at 40 µg

Secondary

Lanes 1 - 2:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Lanes 3 - 4:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 55 kDa, 36 kDa

Exposure time: 180s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.