

### Anti-CD40 antibody [EPR20735] ab224639

KO 評価済 リコンビナント RabMAb

8 References 画像数 11

#### 製品の概要

製品名	Anti-CD40 antibody [EPR20735]
製品の詳細	Rabbit monoclonal [EPR20735] to CD40
由来種	Rabbit
アプリケーション	適用あり: WB, IHC-P, Flow Cyt, IP, ICC/IF
種交差性	交差種: Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: Human lymph node and lymphoma tissue lysates; Raji whole cell lysate. IHC-P: Human tonsil, Hodgkin's lymphoma and diffuse large B-cell lymphoma tissues. ICC/IF: Raji cells. Flow Cyt: Raji cells, Human PBMCs. IP: Raji whole cell lysate. ICC/IF KO: U2OS cells (U2OS-CD40 knockout cells used as negative cell line)
特記事項	<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>

#### 製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR20735

アイソタイプ

IgG

## アプリケーション

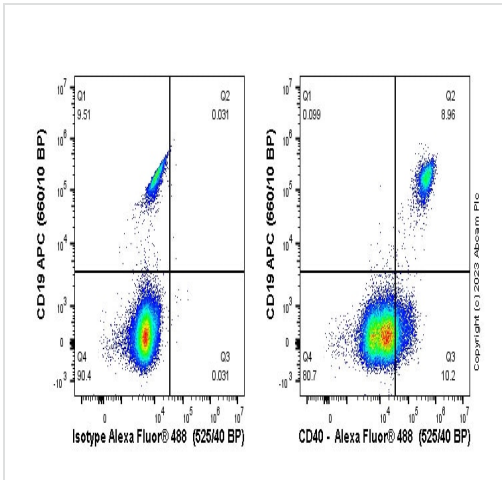
**The Abpromise guarantee** **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab224639の使用に適用されず  
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		1/1000. Detects a band of approximately 42 kDa (predicted molecular weight: 30 kDa).
IHC-P		1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt		1/600. <b>ab172730</b> -Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP		1/30.
ICC/IF		1/100.

## ターゲット情報

機能	Receptor for TNFSF5/CD40LG.
組織特異性	B-cells and in primary carcinomas.
関連疾患	Defects in CD40 are the cause of hyper-IgM immunodeficiency syndrome type 3 (HIGM3) [MIM:606843]; also known as hyper-IgM syndrome 3. HIGM3 is an autosomal recessive disorder which includes an inability of B cells to undergo isotype switching, one of the final differentiation steps in the humoral immune system, an inability to mount an antibody-specific immune response, and a lack of germinal center formation.
配列類似性	Contains 4 TNFR-Cys repeats.
細胞内局在	Secreted and Cell membrane.

## 画像

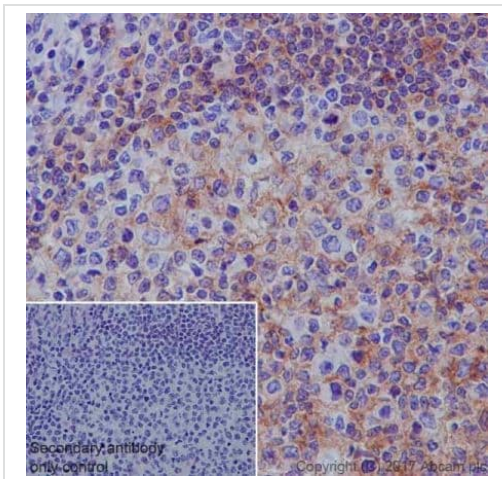


Flow Cytometry - Anti-CD40 antibody [EPR20735] (ab224639)

Flow cytometry staining of human peripheral blood mononuclear cells (PBMCs) with ab224639 (right) or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (left). PBMCs were incubated for 30 min on ice in 1x PBS containing 10 µg/ml human IgG and 10 % normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody ab224639 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control ( $1 \times 10^6$  in 100 µl at 0.2 µg/ml (1/10550)) for 30min on ice. The cells were simultaneously stained with CD19.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min on ice

Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on viable cells.

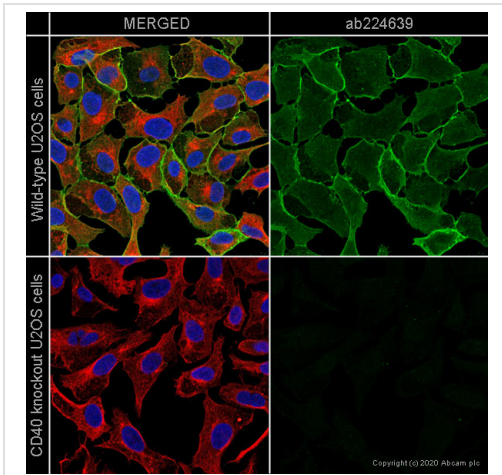


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD40 antibody [EPR20735] (ab224639)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CD40 with ab224639 at 1/250 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Membranous and cytoplasmic staining in human tonsil tissue (PMID:20593977; PMID:7507299) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

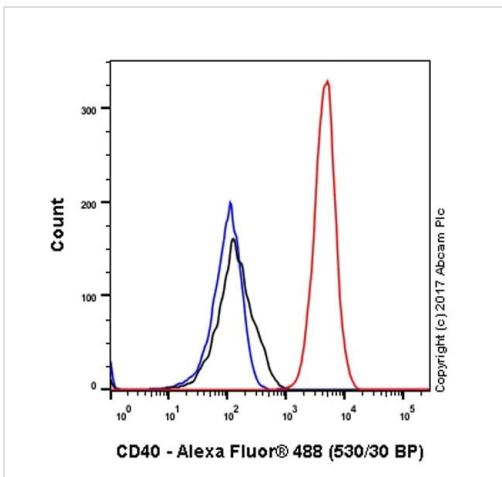
Perform heat mediated antigen retrieval using [ab93684](#) (Tris/EDTA buffer, pH 9.0).



Immunocytochemistry/ Immunofluorescence - Anti-CD40 antibody [EPR20735] (ab224639)

ab224639 staining CD40 in wild-type U2OS cells (top panel) and CD40 knockout U2OS cells (bottom panel). The cells were fixed with PFA (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab224639 at 1/100 dilution and **ab7291** (Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

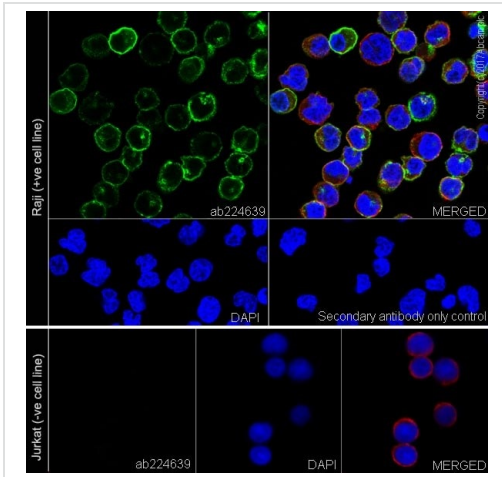
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Flow Cytometry - Anti-CD40 antibody [EPR20735] (ab224639)

Flow cytometric analysis of Raji (human Burkitt's lymphoma cell line) cell line labeling CD40 with ab224639 at 1/600 dilution (red) compared with a 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.

Gated on total viable cells.



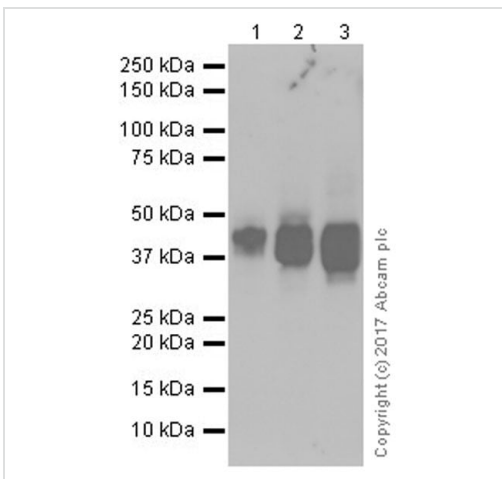
Immunocytochemistry/ Immunofluorescence - Anti-CD40 antibody [EPR20735] (ab224639)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Raji (human Burkitt's lymphoma cell line) cells labeling CD40 with ab224639 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 membranous and weakly cytoplasmic staining in Raji cell line.

**Negative control:** Jurkat T cell line (PMID:10498643).

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: PBS instead of primary antibody, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) ([ab150077](#)) secondary antibody at 1/1000 dilution.



Western blot - Anti-CD40 antibody [EPR20735] (ab224639)

**All lanes :** Anti-CD40 antibody [EPR20735] (ab224639) at 1/1000 dilution

**Lane 1 :** Human lymph node tissue lysate

**Lane 2 :** Human lymphoma tissue lysate

**Lane 3 :** Raji (human Burkitt's lymphoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary**

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Developed using the ECL technique.

**Predicted band size:** 30 kDa

**Observed band size:** 42 kDa

**Exposure time:** 15 seconds

Blocking/Dilution buffer: 5% NFDm/TBST.

CD40 was immunoprecipitated from 0.35 mg of Raji (human Burkitt's lymphoma cell line) whole cell lysate with ab224639 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab224639 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution.

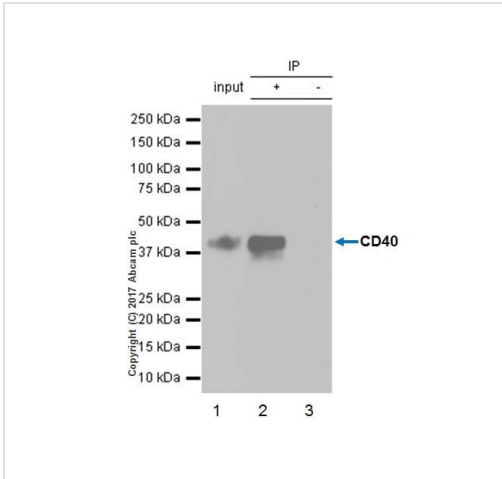
**Lane 1:** Raji whole cell lysate 10 µg (Input).

**Lane 2:** ab224639 IP in Raji whole cell lysate (+).

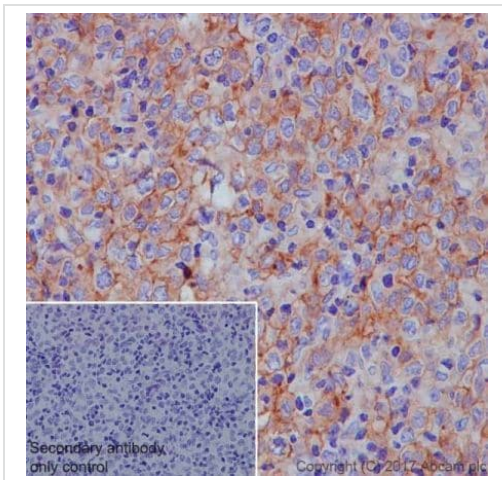
**Lane 3:** Rabbit monoclonal IgG (**ab172730**) instead of ab224639 in Raji whole cell lysate (-).

**Exposure time:** 3 minutes.

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



Immunoprecipitation - Anti-CD40 antibody  
[EPR20735] (ab224639)

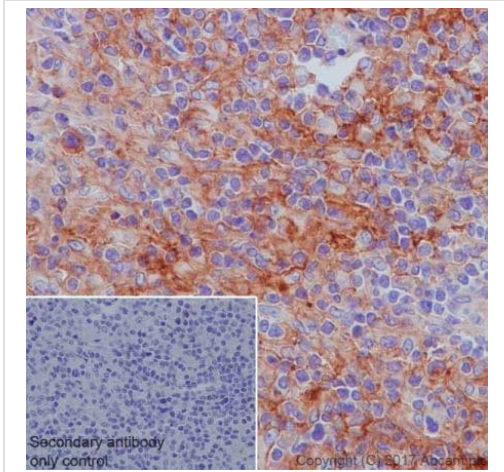


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD40 antibody  
[EPR20735] (ab224639)

Immunohistochemical analysis of paraffin-embedded human diffuse large B-cell lymphoma tissue labeling CD40 with ab224639 at 1/250 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Membranous staining in human diffuse large B-cell lymphoma tissue (PMID: 20593977) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD40 antibody [EPR20735] (ab224639)

Immunohistochemical analysis of paraffin-embedded human Hodgkin's lymphoma tissue labeling CD40 with ab224639 at 1/250 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Membranous and cytoplasmic staining in Reed-Sternberg cells and tumor cells of human Hodgkin's lymphoma (PMID:7507299) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

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

Tissue Microarray (TMA) data for ab224639

Normal tissue samples		Malignant tissue samples	
Human cardiac muscle	x	Human placenta	x
Human cerebrum	x	Human skeletal muscle	x
Human colon	x (immune cells ✓)	Human skin	x
Human endometrium	x	Human spleen	x
Human kidney	x	Human stomach	x (immune cells ✓)
Human liver	x	Human testis	x
Human lung	x	Human thyroid	x
Human mammary gland	x	Human tonsil	✓
Human pancreas	x		
		Clear cell carcinoma of human kidney	x
		Human astrocytoma	x
		Human bladder cancer	x
		Human breast carcinoma	x
		Human cervical carcinoma	x (immune cells ✓)
		Human colon carcinoma	x (immune cells ✓)
		Human diffuse large-B-cell lymphoma	✓
		Human endometrial carcinoma	x (immune cells ✓)
		Human gastric adenocarcinoma	x
		Human Hodgkin's lymphoma	✓
		Human hepatocellular carcinoma	x
		Human lung carcinoma	x (immune cells ✓)
		Human ovarian carcinoma	x
		Human pancreatic carcinoma	x (immune cells ✓)
		Human prostatic hyperplasia	x
		Human thyroid carcinoma	x

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD40 antibody [EPR20735] (ab224639)

Tissue Microarrays stained for "Anti-CD40 antibody [EPR20735]" using "ab224639" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using [ab93684](#) (Tris/EDTA buffer, pH 9.0). The sections were incubated with ab224639 at +4°C overnight followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer).

### Why choose a recombinant antibody?



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**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

Anti-CD40 antibody [EPR20735] (ab224639)

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