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Datasheet for ABIN670371 anti-CD25 antibody (AA 201-268)

1 Validation

2 Images



Overview

Quantity:	100 µL
Target:	CD25 (IL2RA)
Binding Specificity:	AA 201-268
Reactivity:	Human, Mouse
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This CD25 antibody is un-conjugated
Application:	Flow Cytometry (FACS), Western Blotting (WB), ELISA, Immunohistochemistry (Paraffin- embedded Sections) (IHC (p))

Product Details

Immunogen:	KLH conjugated synthetic peptide derived from mouse CD25/IL2RA
lsotype:	lgG
Cross-Reactivity:	Human, Mouse
Predicted Reactivity:	Rat
Purification:	Purified by Protein A.
Target Details	
Target:	CD25 (IL2RA)
Alternative Name:	IL2RA (IL2RA Products)

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Target Details	
Background:	Synonyms: CD25, II2r, Ly-43, Interleukin-2 receptor subunit alpha, IL-2 receptor subunit alpha, IL-
	2-RA, IL-2R subunit alpha, IL2-RA, p55, Il2ra
	Background: The interleukin 2 (IL2) receptor alpha (IL2RA) and beta (IL2RB) chains, together
	with the common gamma chain (IL2RG), constitute the high-affinity IL2 receptor. Homodimeric
	alpha chains (IL2RA) result in low-affinity receptor, while homodimeric beta (IL2RB) chains
	produce a medium-affinity receptor. Normally an integral-membrane protein, soluble IL2RA has
	been isolated and determined to result from extracellular proteolyisis. Alternately-spliced IL2RA
	mRNAs have been isolated, but the significance of each is presently unknown. Mutations in this
	gene are associated with interleukin 2 receptor alpha deficiency.[provided by RefSeq, Nov
	2009].
Gene ID:	16184
UniProt:	P01590
Pathways:	JAK-STAT Signaling, Growth Factor Binding, Activated T Cell Proliferation

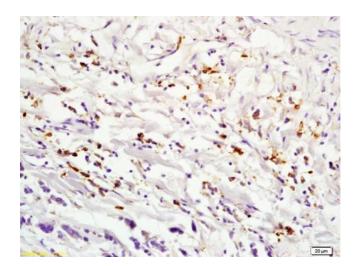
Application Details

Application Notes:	WB 1:300-5000
	ELISA 1:500-1000
	FCM 1:20-100
	IHC-P 1:200-400
Restrictions:	For Research Use only

Handling

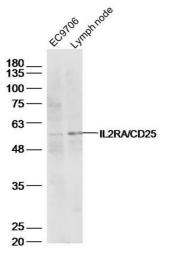
Format:	Liquid
Concentration:	1 µg/µL
Buffer:	0.01M TBS(pH 7.4) with 1 % BSA, 0.02 % Proclin300 and 50 % Glycerol.
Preservative:	ProClin
Precaution of Use:	This product contains ProClin: a POISONOUS AND HAZARDOUS SUBSTANCE, which should be handled by trained staff only.
Storage:	4 °C,-20 °C
Storage Comment:	Shipped at 4°C. Store at -20°C for one year. Avoid repeated freeze/thaw cycles.
Expiry Date:	12 months

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Immunohistochemistry

Image 1. Formalin-fixed and paraffin embedded human lung carcinoma labeled with Anti-IL2RA Polyclonal Antibody, Unconjugated (ABIN670371) at 1:200 followed by conjugation to the secondary antibody and DAB staining



Western Blotting

Image 2. Lane 1: EC9706 lysates Lane 2: mouse lymph node lysates probed with IL2RA Polyclonal Antibody, Unconjugated at 1:500 dilution and 4°C overnight incubation. Followed by conjugated secondary antibody incubation at 1:10000 for 60 min at 37°C.

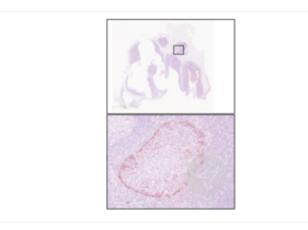
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NDEPENDEN	Successfully validated (Immunohistochemistry (IHC))
Д	by TRON
	Report Number: 104539
VALIDATION	Date: Feb 28 2024
VALIDATION CUSTOMER VALIDATION № DATE 104539 28/02/24	
arget:	CD25
∟ot Number:	AG10271265
Method validated:	Immunohistochemistry (IHC)
Positive Control:	FFPE human tonsil
lotes:	Passed. The CD25 antibody ABIN670371 recognizes CD25 in human tonsil tissue.
Primary Antibody:	ABIN670371
Secondary Antibody:	HRP-conjugated goat anti-rabbit antibody (medac diagnostics, DPVR-110HRP, Lot 101122)
Protocol:	 Dewax and rehydrate tissue sections on glass slides in a descending xylene and graded alcohol series: xylene 2x for 5 min.
	 100 % (v/v) ethanol 2x for 5 min.
	\circ 96 % (v/v) ethanol 2x for 5 min.
	 70 % (v/v) ethanol 2x for 5 min.
	• Wash slides $2x 5$ min with ddH ₂ O.
	 Cook slides for 30 min at 95 °C in citrate buffer pH 6.
	• Wash slides $2x 3 \text{ min with } ddH_2O$.
	• Incubate slides in 0.3 % (v/v) H_2O_2 in PBS for 15 min to quench endogenous peroxidase.
	Wash slides 3x 3 min in 1X PBS.
	Block slides in 10 % goat serum in PBS for 30 min at RT.
	 Incubate slides with primary rabbit anti-CD25 antibody (antibodies-online, ABIN670371, lot AG10271265) diluted 1:250 and 1:500 in 10 % goat serum in PBS ON at 4 °C in a humidified chamber.
	Wash slides 3x 3 min in 1X PBS.
	Incubate slides with secondary HRP-conjugated goat anti-rabbit antibody (medac
	diagnostics, DPVR-110HRP, Lot 101122) ready-to-use in 10 % goat serum in PBS for 30 mi
	at RT in a humidified chamber.
	• Wash slides 3x 3 min in 1X PBS.
	HRP development.
	Counterstain slides with hematoxylin solution (Carl Roth, T865.2).
	Wash slides for 15 min under running tap water.

	 70% ethanol 2x for 10 sec. 96% ethanol 2x for 10 sec. 100% ethanol 2x for 10 sec. xylene 2x for 10 sec. Mount sections with Entellan new mounting medium (Sigma-Aldrich, 1008690500). Dry sections. Image acquisition on an Aperio CS2 Digital Pathology Slide Scanner (Leica), Magnification 40x.
Experimental Notes:	 ABIN670371 appears to successfully recognize CD25 in human tonsils, but in mouse spleen and lung, the signal is not present where expected. ABIN670371 does not appear to recognize CD25 in mouse samples. CD25 should be highly expressed on Tregs, which by definition express FoxP3. ABIN670371 signal does not colocalize with FoxP3 in either spleen or lung. Furthermore, in both tissues, the signal from ABIN670371 is nuclear, whereas it is expected to be membrane. ABIN670371 signal does not co-localize with CD8/CD4 in either spleen or lung although CD25 should (at least partially) co-localize with CD8 and CD4.

Images for Validation report #104539

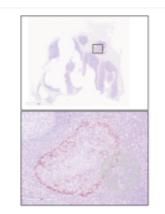


Validation image no. 1 for anti-Interleukin 2 Receptor, alpha (IL2RA) (AA 201-268) antibody (ABIN670371)

IIHC staining of human tonsil with anti-CD25 antibody ABIN670371 (1:250) and counterstain with hematoxylin.



IHC staining of human tonsil with anti-CD25 antibody ABIN670371 (1:500) and counterstain with hematoxylin.



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