abcam

Product datasheet

Donkey Anti-Rabbit IgG H&L (Alexa Fluor® 647) ab150075

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Overview

Product name Donkey Anti-Rabbit IgG H&L (Alexa Fluor® 647)

Host species Donkey

Target species Rabbit

Tested applications Suitable for: ICC/IF, ELISA, IHC-P, Flow Cyt, IHC-Fr

Immunogen The details of the immunogen for this antibody are not available.

Conjugation Alexa Fluor® 647. Ex: 652nm, Em: 668nm

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.

Avoid freeze / thaw cycle. Stable for 12 months at -20°C. Store In the Dark.

Storage buffer Preservative: 0.02% Sodium azide

Constituents: 23% Glycerol (glycerin, glycerine), PBS, 1% BSA

Purity Immunogen affinity purified

Purification notesThis antibody was isolated by affinity chromatography using antigen coupled to agarose beads.

Clonality Polyclonal

Isotype IgG

General notes Alexa Fluor[®] is a registered trademark of Molecular Probes, Inc, a Thermo Fisher Scientific

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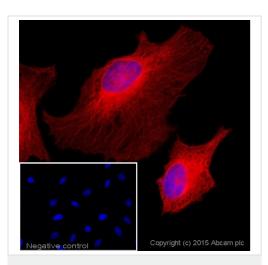
Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab150075 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

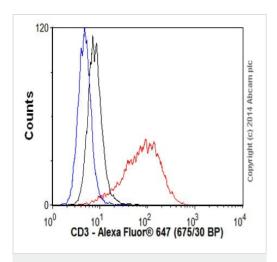
Application	Abreviews	Notes
ICC/IF	**** <u>(1)</u>	1/200 - 1/1000.
ELISA		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration.
Flow Cyt		1/2000 - 1/4000. ab199093 - Rabbit monoclonal lgG (Alexa Fluor® 647), is suitable for use as an isotype control to complement this secondary antibody.
IHC-Fr	****(<u>2</u>)	Use at an assay dependent concentration.

Images

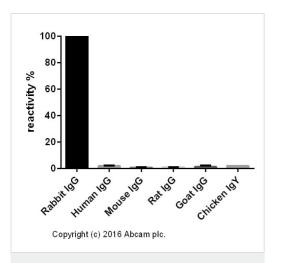


Immunocytochemistry/ Immunofluorescence -Donkey Anti-Rabbit IgG H&L (Alexa Fluor® 647) (ab150075) ICC/IF image of <u>ab6046</u> stained HeLa cells. The cells were 100% formaldehyde fixed (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then incubated in 1%BSA / 10% normal donkey serum / 0.3M glycine in 0.1% PBS-Tween for 1h to block non-specific protein-protein interactions. The cells were then incubated with the primary antibody (<u>ab6046</u>, 5 μ g/ml) overnight at +4°C. The secondary antibody (red) was ab150075 Alexa Fluor® 647 donkey anti-rabbit lgG (H+L) used at 1 μ g/ml for 1h.DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43 μ M.

The negative control (inset) is a secondary-only assay to demonstrate low non-specific binding of the secondary antibody.



Flow Cytometry - Donkey Anti-Rabbit IgG H&L (Alexa Fluor® 647) (ab150075)



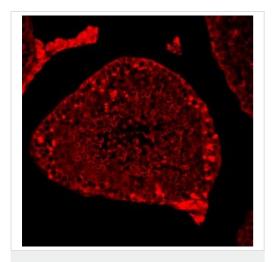
ELISA - Donkey Anti-Rabbit IgG H&L (Alexa Fluor® 647) (ab150075)

Overlay histogram showing Jurkat cells stained with <u>ab16669</u> (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal donkey serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (<u>ab16669</u>, 1/1000 dilution) for 30 min at 22°C. The secondary antibody Donkey anti-rabbit lgG H&L (Alexa Fluor® 647) (ab150075) was used at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monclonal) (0.1µg/1x10⁶ cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a solid-state 25mW red diode laser (635nm) and 675/30 bandpass filter.

Cross-reactivity of the polyclonal secondary antibody <u>ab182020</u> was tested using a sandwich ELISA approach. The wells were coated with the indicated IgG standards at 1 μ g/ml (50 μ l/well) and incubated overnight at 4°C, followed by a 5% BSA blocking step for 2h at RT. <u>ab182020</u> was then added starting at 1 μ g/ml and gradually diluted 1/4 (50 μ l/well), followed by incubation for 2h. For the detection Goat anti-Donkey IgG H&L (HRP) (<u>ab6988</u>) was used at 1/20,000 dilution (50 μ l/well), followed by incubation for 1h at RT.

For the batch tested, <u>ab182020</u> showed a cross-reactivity below 2% towards human IgG, mouse IgG, rat IgG, goat IgG and chicken IgY.

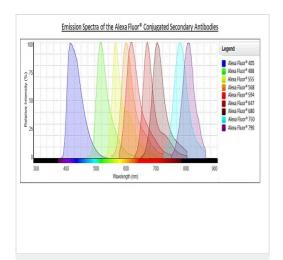
This data was developed using the unconjugated antibody (<u>ab182020</u>).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Donkey Anti-Rabbit IgG H&L (Alexa Fluor® 647) (ab150075)

This image is courtesy of an anonymous Abreview.

ab62050 staining prostaglandin E synthase in mouse testis tissue sections by Immunohistochemistry (IHC-P - paraformaldehydefixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde, permeabilized with Triton X-100, and blocked with 10% serum for 30 minutes at 24°C; antigen retrieval was by heat mediation in a citrate buffer. Samples were incubated with primary antibody (1/50) for 20 hours at 4°C. An Alexa Fluor® 647-conjugated donkey anti-rabbit IgG H&L (ab150075) (1/200) was used as the secondary antibody.



Fluor® 647) (ab150075)

Alexa Fluor® - Donkey Anti-Rabbit IgG H&L (Alexa

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