# abcam

### Product datasheet

## Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) ab150077

\*\*\*\*\* 20 Abreviews 2381 References 16 Images

Overview		
Product name	Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488)	
Host species	Goat	
Target species	Rabbit	
Specificity	This antibody is specific to Rabbit IgG.	
Tested applications	Suitable for: ICC/IF, Flow Cyt, IHC-P, ELISA, IHC-Fr	
Immunogen	The details of the immunogen for this antibody are not available.	
Conjugation	Alexa Fluor® 488. Ex: 495nm, Em: 519nm	
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 notes	This antibody was isolated by affinity chromatography using antigen coupled to agarose beads.	
Clonality	Polyclonal	
lsotype	lgG	
General notes	Fluorochrome chart – a complete guide:	
	A quick and easy guide to help you select the most appropriate fluorochromes for your next experiment.	
	Please see <u>here</u> .	
	Alexa Fluor <sup>®</sup> is a registered trademark of Molecular Probes, Inc, a Thermo Fisher Scientific Company. The Alexa Fluor <sup>®</sup> dye included in this product is provided under an intellectual property license from Life Technologies Corporation. As this product contains the Alexa Fluor <sup>®</sup> dye, the purchase of this product conveys to the buyer the non-transferable right to use the purchased product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). As this product contains the Alexa Fluor <sup>®</sup> dye the sale of this product is expressly conditioned on the buyer not using the product or its components, or any	

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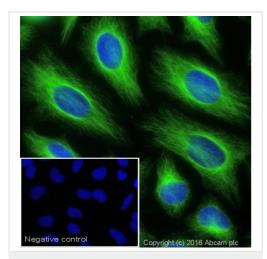
#### **Applications**

 The Abpromise guarantee
 Our Abpromise guarantee
 covers the use of ab150077 in the following tested applications.

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

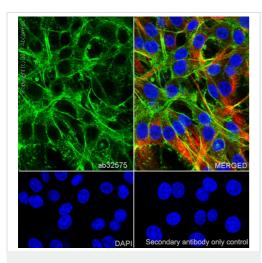
Application	Abreviews	Notes
ICC/IF	$\star$ $\star$ $\star$ $\star$ $\star$ <u>(7)</u>	1/200 - 1/1000.
Flow Cyt		1/2000 - 1/4000.
IHC-P	$\star$ $\star$ $\star$ $\star$ $\star$ (3)	Use at an assay dependent concentration.
ELISA		Use at an assay dependent concentration.
IHC-Fr	$\star$ $\star$ $\star$ $\star$ $\star$ (5)	Use at an assay dependent concentration.

#### Images



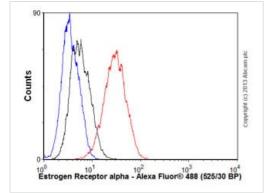
Immunocytochemistry/ Immunofluorescence - Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) ICC/IF image of beta Tubulin staining in HeLa cells. The cells were 100% methanol fixed (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then incubated in 1% BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to block nonspecific protein-protein interactions. The cells were then incubated with the primary antibody (**ab6046**, 5µg/ml) overnight at +4°C. The secondary antibody (green) was ab150077 Alexa Fluor<sup>®</sup> 488 goat anti-rabbit IgG (H+L) used at 2µg/ml for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

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

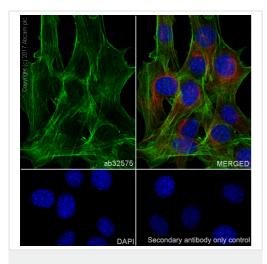


Immunocytochemistry/ Immunofluorescence - Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077)

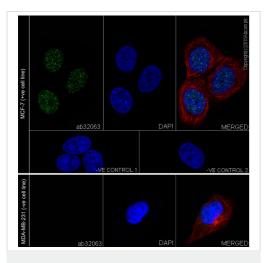
Immunocytochemistry/ Immunofluorescence analysis of C6(Rat glial tumor glial cell) cells labeling alpha smooth muscle Actin with purified **ab32575** at 1/100 dilution (0.71 µg/ml). Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 dilution (2 µg/ml) dilution. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Flow Cytometry (Intracellular) - Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) Overlay histogram showing MCF7 cells stained with unpurified ab32063 (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 goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32063, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) (ab150077) at 1/2000 dilution for 30 min at 22°C. lsotype control antibody (black line) was rabbit IgG (monoclonal) (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 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in MCF7 cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) Immunocytochemistry/ Immunofluorescence analysis of NIH/3T3(Mouse embryonic fibroblast) cells labeling alpha smooth muscle Actin with purified **ab32575** at 1/500 dilution (5.2  $\mu$ g/ml). Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Cells were counterstained with Ab195889 Antialpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5  $\mu$ g/ml). Goat anti rabbit lgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 dilution (2  $\mu$ g/ml) dilution. DAPI was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody antibody antibody only control.

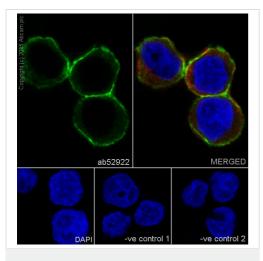


Immunocytochemistry/ Immunofluorescence - Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077)

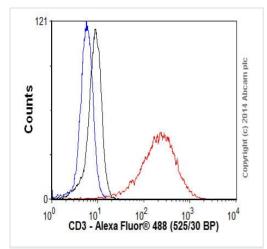
Immunocytochemistry/Immunofluorescence analysis of MCF-7 cells labelling Estrogen Receptor alpha with purified <u>ab32063</u> at 1/1000. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. The cells were co-stained with <u>ab7291</u>, a mouse anti-tubulin (1/1000) using <u>ab150120</u>, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) as the secondary antibody. Nuclei counterstained with DAPI (blue).

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

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



Immunocytochemistry/ Immunofluorescence - Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077)



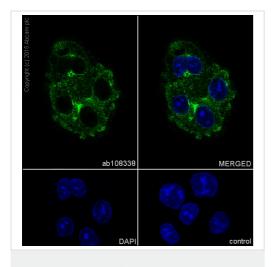
Flow Cytometry - Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077)

Immunocytochemistry/Immunofluorescence analysis of Raji (human Burkitt's lymphoma) cells labelling HLA A with purified **ab52922** at 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, <u>ab150120</u>, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

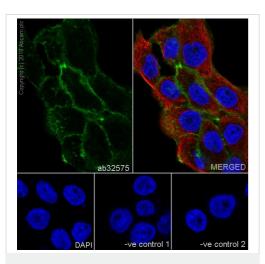
Control 2: <u>**ab7291**</u> (1/1000) and secondary antibody, ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit lgG (1/500).

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 goat 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 Goat anti-rabbit IgG H&L (Alexa Fluor® 488) (ab150077) was used at 1/4000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monclonal) ( $0.1\mu g/1x10^6$  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 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

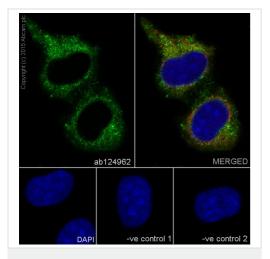


Immunocytochemistry/ Immunofluorescence analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling ATG9A with Purified **ab108338** at 1/100 dilution. Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. ab150077 Goat anti rabbit IgG (Alexa Fluor® 488) was used as the secondary antibody at 1/1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

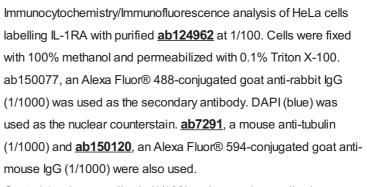
Immunocytochemistry/ Immunofluorescence - Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077)



Immunocytochemistry/ Immunofluorescence - Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) Immunocytochemistry/Immunofluorescence analysis of A431 (human epidermoid carcinoma) cells labeling alpha smooth muscle Actin (green) with purified <u>ab32575</u> at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. Cells were counterstained with <u>ab7291</u>, anti-Tubulin (mouse mAb) at 1/1000 followed by <u>ab150120</u> Alexa Fluor®594 goat anti-mouse secondary (1/1000). Nuclei were counterstained with DAPI (blue). For negative control 1, rabbit primary antibody and anti-mouse secondary antibody (<u>ab150120</u>) were used. For negative control 2, <u>ab7291</u> (mouse primary antibody) was used followed by anti-rabbit secondary antibody (ab150077).

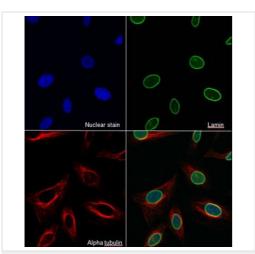


Immunocytochemistry/ Immunofluorescence - Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077)



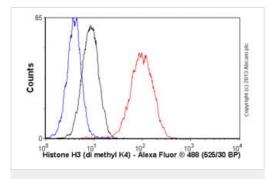
Control 1: primary antibody (1/100) and secondary antibody, <u>ab150120</u>, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

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



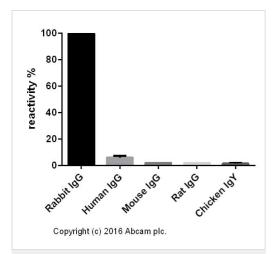
Immunocytochemistry/ Immunofluorescence - Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077)

The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (**ab7291**, 1µg/ml) and (**ab16048**, 1µg/ml) overnight at +4°C. The secondary antibodies were **ab150115** Alexa Fluor® 647 (red) goat anti-mouse lgG (H+L) used at 2µg/ml for 1h and ab150077 Alexa Fluor® 488 (green) goat anti-rabbit lgG (H+L) used at 2µg/ml for 1h. DAPI was used to stain the cell nuclei.

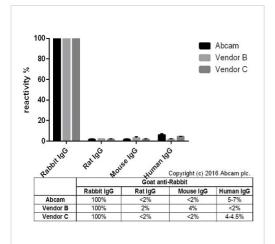


Flow Cytometry - Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077)

Overlay histogram showing HeLa cells stained with **ab32356** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (**ab32356**, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 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 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



ELISA - Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077)



ELISA - Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077)

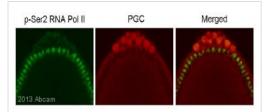
Cross-reactivity of the polyclonal secondary antibody <u>ab182016</u> was tested using a sandwich ELISA approach. The wells were coated with the indicated IgG standards at 1 µg/ml (50 µl/well) and incubated overnight at 4°C, followed by a 5% BSA blocking step for 2h at RT. <u>ab182016</u> was then added starting at 1 µg/ml and gradually diluted 1/4 (50 µl/well), followed by incubation for 2h. For the detection Donkey anti-Goat IgG H&L (HRP) (<u>ab6885</u>) was used at 1/10,000 dilution (50 µl/well), followed by incubation for 1h at RT.

For the batch tested, <u>ab182016</u> showed a cross-reactivity of 5-7% towards Human IgG and below 2% towards Mouse IgG, Rat IgG and Chicken IgY.

This data was developed using the unconjugated antibody (<u>ab182016</u>).

Cross-reactivity of Goat anti-Rabbit IgG H&L (**ab182016**) and Goat anti-Rabbit IgG H&L obtained from two different vendors was tested using a sandwich ELISA approach. The wells were coated with the indicated IgG standards (Rabbit, Human, Mouse and Rat) at 1  $\mu$ g/ml (50  $\mu$ I/well) and incubated overnight at 4°C, followed by a 5% BSA blocking step for 2h at RT. Secondary antibodies were then added starting at 1  $\mu$ g/ml and gradually diluted 1/4 (50  $\mu$ I/well), followed by incubation for 2h. For the detection Donkey anti-Goat IgG H&L (HRP) (**ab6885**) was used at 1/10,000 dilution (50  $\mu$ I/well), followed by incubation for 1h at RT. This data is from a representative dilution.

This data was developed using the unconjugated antibody (ab182016).



IHC - Wholemount - Goat Anti-Rabbit IgG H&L

(Alexa Fluor® 488) (ab150077)

This image is courtesy of an anonymous Abreview.

Emission Spectra of the Alexa Fluor<sup>®</sup> Conjugated Secondary Antibodies The Alexa Fluor<sup>®</sup> - Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077)

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IHC - Wholemount of Caenorhabditis elegans larvae labelling RNA polymerase II CTD repeat YSPTSPS (phospho S2) with **ab5095**. The sample was incubated with primary antibody (1/500 in PBS + 3% BSA + 0.1% Triton X-100) for 12 hours at 4°C. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG polyclonal (1/1000), was used as the secondary antibody.