abcam

Product datasheet

Alexa Fluor® 647 Conjugation Kit (Fast) - Lightning-Link® ab269823

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Overview

Product name Alexa Fluor® 647 Conjugation Kit (Fast) - Lightning-Link®

Product overview Alexa Fluor® 647 Conjugation Kit / Alexa Fluor® 647 Labeling Kit ab269823 uses a simple and quick process for Alexa Fluor® 647 labeling / conjugation of antibodies. It can also be used to conjugate other proteins or peptides. Learn about our **antibody labeling kits and their advantages**.

To conjugate an antibody to Alexa Fluor® 647 using this kit:
- add modifier to antibody and incubate for 15 mins
- add quencher and incubate for 5 mins
The Alexa Fluor® 647 conjugated antibody can be used immediately in WB, ELISA, IHC etc. No further purification is required and 100% of the antibody is recovered for use.

Learn about buffer compatibility below; for incompatible buffers and low antibody concentrations, use our rapid **antibody purification and concentration kits**. Use the **FAQ** to learn more about the technology, or about conjugating other proteins and peptides to Alexa Fluor® 647.

Custom size conjugation kits up to 100 mg are available on demand. Please contact us to discuss your requirements.

Notes This product is manufactured by Expedeon, an Abcam company, and was previously called Lightning-Link® Rapid Alexa Fluor® 647 Labeling Kit. 337-0005 is the same as the 100 µg size. 336-0010 is the same as the 3 x 100 ug size. 336-0030 is the same as the 3 x 10 ug size. 336-0005 is the same as the 100 µg size. 336-0015 is the same as the 1 mg size.

Amount and volume of antibody for conjugation to Alexa Fluor® 647

Kit size	*Recommended amount of antibody[1]*	*Maximum amount of antibody*	*Maximum antibody volume[2]*
3 x 10 µg	3 x 10 µg	3 x 20 µg	3 x 10 µL
100 µg	100 µg	200 µg	100 µL
3 x 100 µg	3 x 100 µg	3 x 200 µg	3 x 100 µL

1 mg	1 x 1 mg	1 x 2 mg	1 x 1 mL

[1] Using the maximum amount of antibody may result in less labelling per antibody.

[2] Ideal antibody concentration is 1mg/ml. 0.5 - 1 mg/ml can be used if the maximum antibody volume is not exceeded. Antibodies > 2mg/ml or < 0.5 mg/ml should be diluted /concentrated.

Buffer Requirements for Conjugation

Buffer should be pH 6.5-8.5.

Compatible buffer constituents

If a concentration is shown, then the constituent should be no more than the concentration shown. If several constituents are close to the limit of acceptable concentration, then this can inhibit conjugation.

50mM / 0.6% Tris[1]	0.1% BSA[2]	50% glycerol
0.1% sodium azide	PBS	Potassium phosphate
Sodium chloride	HEPES	Sucrose
Sodium citrate	EDTA	Trehalose

[1] Tris buffered saline is almost always ≤ 50 mM / 0.6%

[2] BSA can also interfere with the use of the conjugated antibody in tissue staining.

Incompatible buffer constituents

Thiomerosal	Proclin	Glycine
Arginine	Glutathione	DTT

If a constituent of the buffer containing your antibody or protein is not listed above, please check the **FAQ** or **contact us**.

Only purified antibodies are suitable for use, ie. where other proteins, peptides, or amino acids are not present: antibodies in ascites fluid, serum or hybridoma culture media are incompatible.

Storing and handling conjugation kits

Lyophilized Lightning-Link® components are hygroscopic.

Kits are intentionally shipped at ambient temperature with silica gel to avoid exposure to moisture. Upon receipt, store the kit frozen and protect from moisture. Before opening the outer container, allow the lyophilized components to reach room temperature to minimize condensation.

Alexa Fluor® is a registered trademark of Molecular Probes, Inc, a Thermo Fisher Scientific Company. The Alexa Fluor® dye included in this product is provided under an intellectual property license from Life Technologies Corporation. As this product contains the Alexa Fluor® 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® dye the sale of this product is expressly conditioned on the buyer not using the product or its components, or any materials made using the product or its components, in any activity to generate revenue, which may include, but is not limited to use of the product or its components: in manufacturing; (ii) to

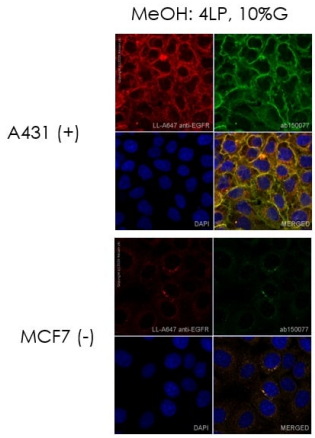
provide a service, information, or data in return for payment (iii) for therapeutic, diagnostic or prophylactic purposes; or (iv) for resale, regardless of whether they are sold for use in research. For information on purchasing a license to this product for purposes other than research, contact Life Technologies Corporation, 5781 Van Allen Way, Carlsbad, CA 92008 USA or **outlicensing@thermofisher.com**.

Properties

Storage instructions Store at -20°C. Please refer to protocols.

Components	100 µg	1 mg	3 x 10 µg	3 x 100 µg
ab274049 - Alexa Fluor 647	1 x 100µg	1 x 1mg	3 x 10µg	3 x 100µg
ab273994 - Modifier reagent	1 x 200µl	1 x 200µl	1 x 200µl	1 x 200µl
ab273995 - Quencher reagent	1 x 200µl	1 x 200µl	1 x 200µl	1 x 200µl

Images



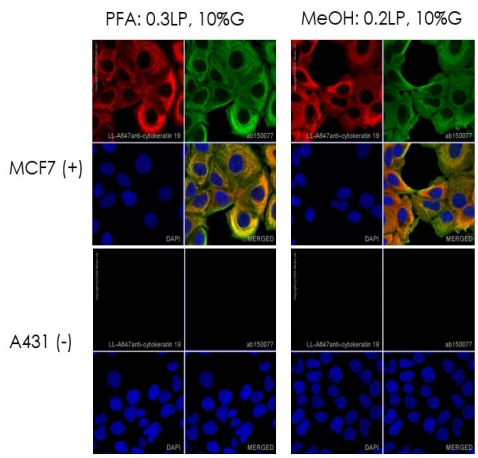
Direct immunofluorescence - Lightning-Link® Alexa Fluor® 647 Anti-EGFR antibody [E235]

Direct immunofluorescent staining using Recombinant Anti-EGFR antibody [E235] - BSA and Azide free (**ab227459**) labelled with Alexa Fluor® 647 Conjugation Kit (Fast) - Lightning-Link® (**ab269823**).

Lightning-Link® Alexa Fluor® 647 Anti-EGFR antibody [E235] conjugate was used to stain wild-type A431 cells (top panel) and MCF7 negative cells (bottom panel). The cells were fixed with 100% methanol at -20°C (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were incubated with 1 µg/ml of LL-Anti-EGFR Alexa Fluor® 647 conjugate (shown in red) or **ab227459** (Anti-EGFR antibody, unlabelled control) overnight at +4°C. **Ab227459** treated cells only were incubated with **ab150077** at 1/1000 dilution for 1 hour at room temperature (shown in green).

Nuclear DNA was labelled in blue with DAPI.

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



Direct immunofluorescence - Lightning-Link® Alexa Fluor® 647 Anti-Cytokeratin 19 antibody [EP1580Y]

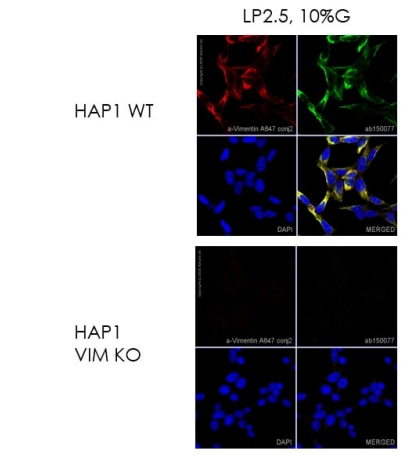
Direct immunofluorescent staining using Recombinant Anti-Cytokeratin 19 antibody [EP1580Y] - BSA and Azide free (**ab195872**) labelled with Alexa Fluor® 647 Conjugation Kit (Fast) - Lightning-Link® (**ab269823**).

Lightning-Link® Alexa Fluor® 647 Anti-Cytokeratin 19 [EP1580Y] conjugate was used to stain wild-type MCF7 cells (top panel) and A431 negative cells (bottom panel).

The cells were fixed with 4% paraformaldehyde (10 min) or 100% methanol at -20°C (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were incubated with 1 µg/ml of LL-Anti-Cytokeratin 19 Alexa Fluor® 647 conjugate (shown in red) or **ab195872** (Anti-Cytokeratin 19 antibody, unlabelled control) overnight at +4°C. **Ab195872** treated cells only were incubated with **ab150077** at 1/1000 dilution for 1 hour at room temperature (shown in green).

Nuclear DNA was labelled in blue with DAPI.

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



Direct immunofluorescence - Lightning-Link® Alexa Fluor® 647 Anti-Vimentin antibody [EPR3776]

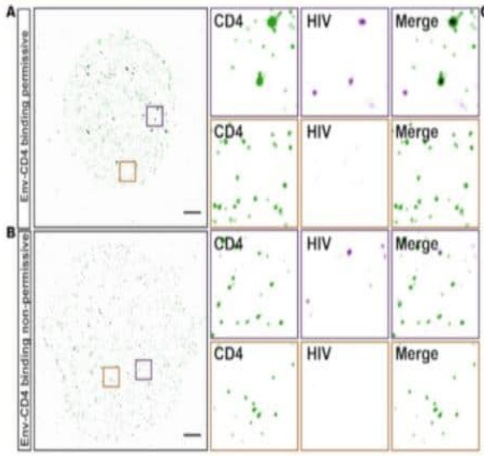
Direct immunofluorescent staining using Recombinant Anti-Vimentin antibody [EPR3776] - BSA and Azide free (**ab193555**) labelled with Alexa Fluor® 647 Conjugation Kit (Fast) - Lightning-Link® (**ab269823**).

Lightning-Link® Alexa Fluor® 647 Anti-Vimentin antibody [EPR3776] conjugate was used to stain Vimentin-wild-type HAP1 cells (top panel) and Vimentin-knock-out HAP1cells (bottom panel). The cells were fixed with 100% methanol at -20°C (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were incubated with 1 µg/ml of LL-Anti-Vimentin Alexa Fluor® 647 (shown in red) or **ab193555** (Anti-Vimentin antibody, unlabelled control) overnight at +4°C. **Ab193555** treated cells only were incubated with **ab150077** at 1/1000 dilution for 1 hour at room temperature (shown in green).

Nuclear DNA was labelled in blue with DAPI.

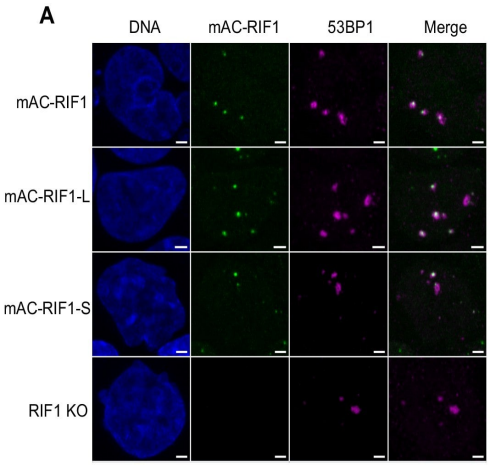
Image was taken with a confocal microscope (Leica-Microsystems,

TCS SP8).

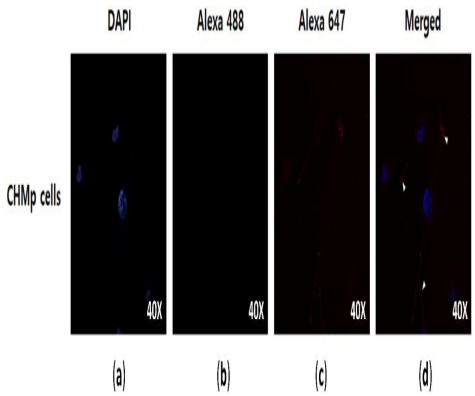


Conjugation - Alexa Fluor® 647 Conjugation Kit (Fast) - Lightning-Link® (ab269823)

Yuan, Yue, et al used Alexa Fluor 647 Conjugation Kit (Fast) - Lightning-Link (ab269823) as part of examining changes in the nanoscale organisation of CD4 on the surface of CD4+ T cells following HIV-1 binding. They used the kit to conjugate Alexa Fluor 647 to anti-CD4 antibody (OKT4) for use in single-molecule super-resolution imaging.
Representative TIRF-STORM images, and selected magnified regions (insets) of cell-surface CD4 (green) and HIV p24 (magenta). Scale bar = 2 ?m.

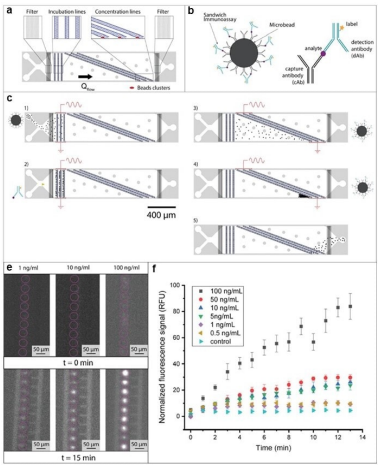


Alexa Fluor® 647 Conjugation Kit (Fast)



Immunocytochemistry - Alexa Fluor 647 Conjugation Kit (Fast) Lightning-Link (ab269823)

Kim, Jin-Wook et al. used Alexa Fluor® 647 Conjugation Kit Lightning-Link® as part of examining alterations in metastatic capacity through cathepsin A by leptin. They used the kit to conjugate anti-LAMP2a antibody for use in immunocytochemistry. Confocal microscopy images of negative control CHMp cells. (a) DAPI stained CHMp cells; (b) Negative control image of Alexa 488?conjugated secondary antibody. (c,d) Negative control image of Alexa 647 - conjugated LAMP2a antibodies. The white arrow? heads indicate the location of LAMP2a only. The images are captured under 40X confocal microscope.



Immunoelectrophoresis - Alexa Fluor Conjugation Kit (Fast)- Lightning-Link (ab269823)

Thiriet, Pierre-Emmanuel, et al used Alexa Fluor® 647 Conjugation Kit (Fast) - Lightning-Link® (ab269823) as part of rapid and accurate diagnosis of Acute kidney injury (AKI). They used the kit to conjugate Alexa Fluor® 647 to monoclonal anti-Lipocalin-2/NGAL antibody for use in the development of a microfluidic analytical device .

(a-c) Chip description and operation. (a) Presentation of the chip layout. Beads and reagents can be successively injected through the two inlets visible on the left. The device consists of three incubation lines upstream and three concentration lines downstream, at the end of which the beads are accumulated in clusters (shown here in red). (b) Illustration of a sandwich immunoassay used for detection of biomarkers. The analyte we aimed to detect was captured by the bead decorated with capture antibody (cAb) and detection was performed thanks to the fluorescently labeled detection antibody (dAb). (c) Presentation of the successive steps performed on-chip to operate the platform, namely, (1) beads' loading, (2) incubation with detection antibodies

and (3) release from the incubation line, (4) clustering in the concentration region, and (5) discarding through the outlet. For the sake of clarity, the species bound to the beads and the electrically activated arrays of electrodes are indicated for each step. (d-f) On-chip incubation of Neutrophil Gelatinase-Associated Lipocalin (NGAL) biomarker. (d) Observation of the small beads' clusters (circled in pink) before and after 15 min of incubation. The fluorescence signal arose from the binding of dAb-NGAL complex to cAb-decorated beads dielectrically trapped in the regions upstream to the electrode line. Three NGAL concentrations were injected in separate experiments, namely, 1 ng/mL, 10 ng/mL, and 100 ng/mL. (f) Fluorescence signal as a function of the incubation time for different NGAL concentrations. After 15 min all concentrations provided a signal greater than the control experiment, consisting of an injection of a solution in absence of NGAL molecules. The error bars were obtained by measuring the fluorescent signal from 10 clusters.

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