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Product datasheet

Cy5® Conjugation Kit (Fast) - Lightning-Link® ab188288

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製品の概要

製品名

Cy5® Conjugation Kit (Fast) - Lightning-Link®

製品の概要

Cy5® Conjugation Kit / Cy5® Labeling Kit (ab188288) uses a simple and quick process for Cy5 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 Cy5 using this kit:
- add modifier to antibody and incubate for 15 min
- add quencher and incubate for 5 mins
The Cy5 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 Cy5.

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

特記事項

This product is manufactured by Expedeon, an Abcam company, and was previously called Lightning-Link® Rapid Cy5 Labeling Kit. 342-0005 is the same as the 100 µg size. 342-0010 is the same as the 3 x 100 ug size. 342-0030 is the same as the 3 x 10 ug size. 342-0015 is the same as the 1 mg size.

Amount and volume of antibody for conjugation to Cy5

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	1 x 100 µg	1 x 200 µg	1 x 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.

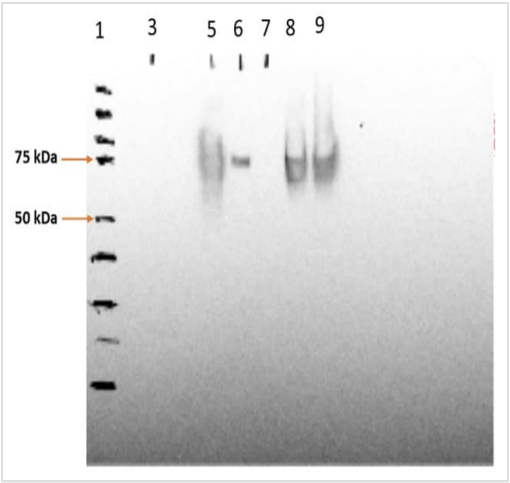
Kits are intentionally shipped at ambient temperature with silica gel to avoid exposure to moisture. Upon receipt, store the kit frozen and protect from moisture. Before opening the outer container, allow the lyophilized components to reach room temperature to minimize condensation.

製品の特性

保存方法 Store at -20°C. Please refer to protocols.

内容	1 mg	100 µg	3 x 10 µg	3 x 100 µg
ab274058 - Cyanine Dye5	1 x 1mg	1 x 100µg	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

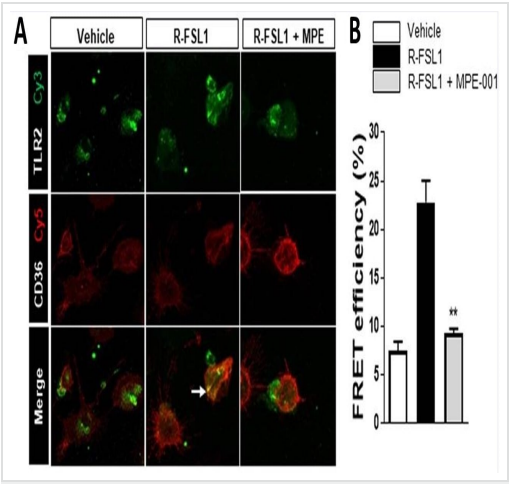
画像



Western blot - Cy5® Conjugation Kit (Fast) - Lightning-Link® (ab188288)

Image from Mody et al., Front bioeng biotechnol., 8:606652; doi: 10.3389/fbioe.2020.606652. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

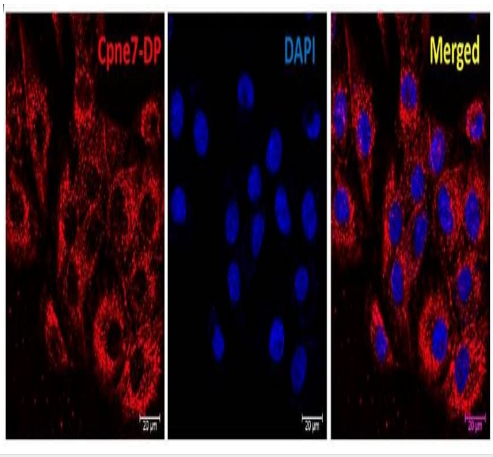
Mody, Karishma T., et al used Cy5® Conjugation Kit (Fast) - Lightning-Link® (ab188288) as part of examining the integrity of Bm86 protein postlabeling. They used the kit to conjugate Cy5® to R. micr-protein ladder, lane 5-Cy5-Bm86 protein, lane 7-Cy5-Bm86/Rho-SV-140-C18 Supernatant, lane 8-Cy5-Bm86/Rho-SV-140-C18 pellet (first antibody 869 polyclonal sheep 1/5,000; second antibody monoclonal anti-goat/sheep 1/10,000). The Cy5-labeled Bm86 retained its native antigenicity as it was recognized by the antibodies in serum from a sheep immunized with the unlabeled antigen from a previous study.



FRET - Cy5® Conjugation Kit (Fast) - Lightning-Link® (ab188288)

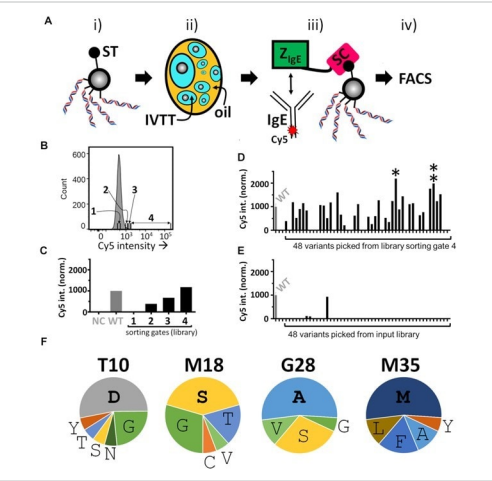
Image from Mellal et al., Sci rep., 9(1):12903. doi: 10.1038/s41598-019-49472-8. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

Mellal, Katia, et al used Cy5® Conjugation Kit (Fast) - Lightning-Link® (ab188288) as part of examining effects of MPE-001 on the CD36/TLR2 interaction. They used the kit to conjugate Cy5® to anti-CD36 antibody (**ab80080**) for use in FRET.
Peritoneal MPs were stimulated with 300 ng/ml R-FSL1 in the presence of 10-7 M MPE-001 or vehicle. (A) MPE-001 disrupted the interaction between CD36 labeled with Cy5 (red) using the Cy5® Conjugation Kit (Fast) - Lightning-Link® (ab188288) and TLR2 labeled with Cy3 (green) using the Cy3® Conjugation Kit (Fast) - Lightning-Link® (**ab188287**) as assessed by FRET after 5 min stimulation with R-FSL1. (B) Percentage of energy transfer measured using LSM-700 confocal microscope (Zeiss). One-way ANOVA test with Newman-Keuls post-test for multiple comparison was performed. *P<0.05, **P



Immunocytochemistry - Cy5® Conjugation Kit (Fast) - Lightning-Link® (ab188288)

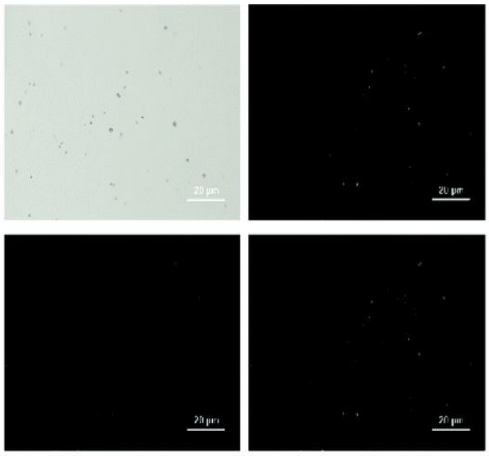
Lee, Yoon Seon, et al used Cy5® Conjugation Kit (Fast) - Lightning-Link® (ab188288) as part of examining the translocation and localization of newly developed peptide derived from CPNE7 (Cpne7-DP) . They used the kit to conjugate Cy5® to Cpne7-DP oligopeptide for use in immunocytochemistry.
The intracellular distribution of Cpne7-DP is shown after odontoblastic MDPC-23 cells were treated with Cy5-labeled Cpne7-DP (10 µg/mL).



Flow Cytometry - Cy5 Conjugation Kit (Fast) Lightning-Link (ab188288)

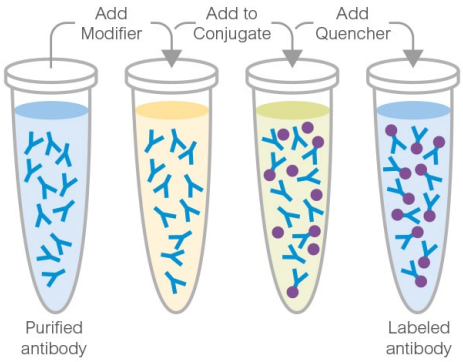
Lindenburg, Laurens, et al used Cy5® Conjugation Kit (Fast) - Lightning-Link® (ab188288) as part of examining microemulsion-based bead display screening of the ZIgE SpliMLiB library. They used the kit to conjugate Cy5® to Native human IgE protein (Azide free) (**ab65866**) for use in FACS.
(A) Schematic overview of a round of SpliMLiB-enabled directed evolution of ZIgE. SpliMLiB beads (i) were singly encapsulated in emulsion IVTT at 37°C for 1 h (ii), sufficient time to allow for both ZIgE-SpyCatcher variants' expression as well as for their SpyTag-SpyCatcher-mediated immobilisation on the bead surface, after which the emulsion was broken, and the washed beads were exposed to Cy5-labelled IgE (iii), followed by flow cytometric sorting of beads based on Cy5 signal (iv). (B) Representative histogram recorded during the flow cytometric sorting of SpliMLiB ZIgE library beads. The range of fluorescence intensity used for each of the sorting gates 1-4 is indicated. (C) Analysis of pooled, recovered and subcloned DNA from the sorting gates set out in panel B. DNA was used to express protein in IVTT under bulk, i.e. non-emulsion conditions, in the presence of SpyTag-functionalised microbeads. The microbeads, having captured the SpyCatcher fusion proteins, were then incubated with 200 nM IgE-Cy5 and analysed by flow

cytometry. Cy5 fluorescence intensity was normalised to a sample prepared from beads exposed to purified ZIgEwild-type-SpyCatcher protein (WT, grey bar). Negative control (NC) was beads not exposed to any ZIgE-SpyCatcher protein. (D) Analysis of bacterially expressed & purified variants derived from the stringently sorted library output from FACS sorting gate 4. Beads that had been bound with ZIgE-SpyCatcher variants were incubated with 200 nM IgE-Cy5 and analysed by flow cytometry. ZIgEwild-type-SpyCatcher (labelled WT) was included as control and was used to normalise all fluorescent values. The variant showing the highest Cy5 median signal (variant 33, marked by a single asterisk) and second highest (variant 44, marked by a double asterisk) signal were taken forward for further analysis. (E) As panel D, except for 48 randomly picked clones derived from the unsorted SpliMLiB input library beads. (F) Frequencies of amino acids encountered in selected variants displaying a higher binding signal than ZIgEwild-type-SpyCatcher (17 in total). The most frequent amino acid at each position is indicated in bold to emphasise it.

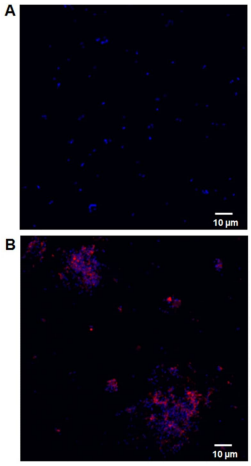


Fluorescence Microscopy - Cy5® Conjugation Kit (Fast) - Lightning-Link® (ab188288)

Li, Tiankuan, et al used Cy5® Conjugation Kit (Fast) - Lightning-Link® (ab188288) as part of examining immunotherapeutical treatments for lung cancer. They used the kit to conjugate Cy5® to anti-PD-L1 mAb for use in fluorescence microscopy.
CLSM images of docetaxel and CY5-labelled anti-PD-L1 mAb-co-loaded microbubbles with a shell containing FITC, scale bar = 20 μm.



conjugation



Cy5® Conjugation Kit - Lightning-Link® labeling

NK2A peptide for confocal microscopy

Image from Dassanayake RP et al., PloS One, 12(8):e0183610. Fig 4.; doi:10.1371/journal.pone.0183610. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

Dassanayake, Rohana P et al used ab188288 as part of examining the antimicrobial activity of bovine NK-lysin-derived peptides.

They used the kit to conjugate Cy5® to NK2A peptide for use in confocal microscopy.

H. somni was incubated with unconjugated Cy5 (A) or 20 µM NK2A-Cy5 conjugate for 30 min and bacterial nuclear contents were stained with DAPI. NK2A-Cy5 conjugate is shown in red and DAPI is shown in blue.

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