

PE/Cy5® Conjugation Kit - Lightning-Link® ab102893

[18 References](#) [7 Images](#)

Overview

Product name PE/Cy5® Conjugation Kit - Lightning-Link®

Product overview PE/Cy5® Conjugation Kit / PE/Cy5® Labeling Kit **ab102871** uses a simple and quick process for PE/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 PE/Cy5® using this kit:

- add modifier to antibody and incubate for 3 hrs
- add quencher and incubate for 30 mins

The PE/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 PE/Cy5®.

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® R-PE/Cy5 Labeling Kit. 760-0005 is the same as the 60 µg size. 760-0010 is the same as the 3 x 60 µg size. 760-0030 is the same as the 3 x 10 µg size. 760-0015 is the same as the 600 µg size.

Amount and volume of antibody for conjugation to PE/Cy5®.

| <i>Kit size</i> | <i>Recommended amount of antibody</i> | <i>Maximum antibody volume¹</i> |
|-----------------|---------------------------------------|--|
| 3 x 10 µg | 3 x 10 µg | 3 x 10 µL |
| 60 µg | 1 x 60 µg | 1 x 60 µL |
| 3 x 60 µg | 3 x 60 µg | 3 x 60 µL |
| 600 µg | 1 x 600 µg | 1 x 600 µL |

¹Ideal antibody concentration is 1mg/ml. 0.5 - 1 mg/ml can be used if the maximum antibody

volume is not exceeded. Antibodies > 1 mg/ml or < 0.5 mg/ml should be diluted /concentrated.

The selling size of this product has been changed – it is now based on the amount of antibody that can be conjugated with the kit, not the amount of PE mix provided. The amount of antibody advised that can be used with the kit has also been updated to reflect what will give the best conjugation results. The quantity and formulation of reagents provided have not changed, if you have been previously using the kit successfully with a different amount of antibody, there is no need to change the way that you are using the kit.

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 | 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%

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.

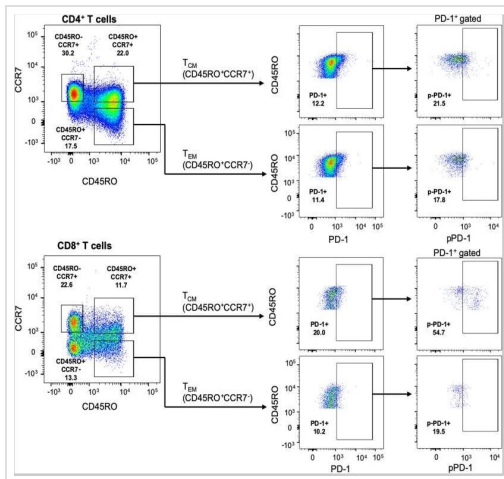
Properties

Storage instructions

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

| Components | 600 µg | 60 µg | 3 x 10 µg | 3 x 60 µg |
|-----------------------------|-----------|-----------|-----------|-----------|
| ab274106 - Modifier reagent | 1 x 200µl | 1 x 200µl | 1 x 200µl | 1 x 200µl |
| ab274144 - PE/Cy5 mix | 1 x 600µg | 1 x 60µg | 3 x 10µg | 3 x 60µg |
| ab274133 - Quencher reagent | 1 x 200µl | 1 x 200µl | 1 x 200µl | 1 x 200µl |

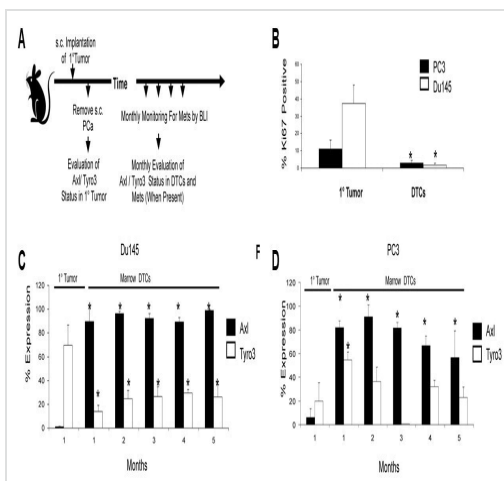
Images



Flow Cytometry - PE/Cy5 Conjugation Kit -
Lightning-Link(ab102893)

Image from Bardhan, Kankana, et al., Scientific reports, 9(1):17252. doi: 10.1038/s41598-019-53463-0 .Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

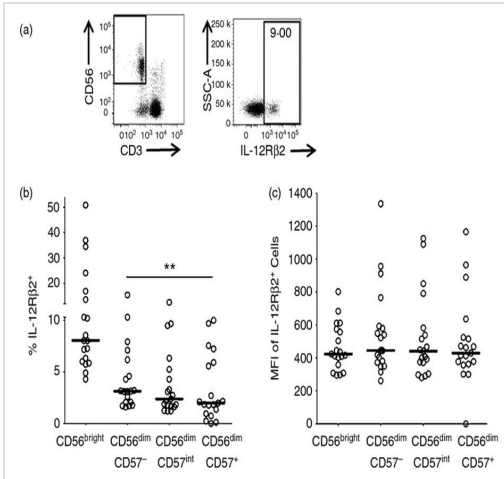
Bardhan, Kankana, et al used PE/Cy5® Conjugation Kit - Lightning-Link® (ab102893) as part of examining PD-1pY248+ (pPD-1) expression in human T cells. They used the kit to conjugate PE/Cy5® to Anti-PD-1pY248 antibody for use in flow cytometry. pPD-1 is predominantly expressed in CD8+ TCM cells. CCR7 and CD45RO markers were used to identify central memory (TCM) and effector memory (TEM) T cells. After gating on CD45RO CD4+ and CD8+ T cells were identified by assessing expression of CCR7. In TCM and TEM populations, expression of PD-1 was determined and, subsequently, expression of pPD-1 (pPD-1-Y248) was assessed in the PD-1+ population within each subset. Results are representative of six separate experiments.



Flow Cytometry - PE/Cy5 Conjugation Kit- Lightning-Link(ab102893)

Image from Taichman, Russell S., et al., PLoS One; 8(4): e61873; doi: 10.1371/journal.pone.0061873. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

Taichman, Russell S., et al used PE/Cy5® Conjugation Kit - Lightning-Link® (ab102893) as part of examining Axl and Tyro3 expression during experimental prostate cancer (PCa) progression. They used the kit to conjugate PE/Cy5® to antibodies for use in flow cytometry. Anti-Axl, anti-Tyro3 and anti-Ki67 antibodies were conjugated to the fluorophores APC-Cy7, PE-Cy5, and Atto390 using our Lightning-Link® Conjugation kits. (A) Experimental model. Human PCa cell lines (PC3Luc, DU145Luc) were implanted s.c. into male SCID mice as a model of a primary (1°) tumor development, and removed after 1 month. At monthly intervals thereafter human PCa cells were identified by anti-HLA staining; proliferative status (Ki67 staining) and Axl or Tyro3 levels were evaluated by FACS. (B) Percent expression of Ki67 by lineage depleted (Lin-) marrow cells or by primary tumor cells at 1 month. (C-D) Percent expression of Axl or Tyro3 by primary tumor cells established with (C) DU145 or (D) PC3 cells or by DTCs recovered from marrow over time.

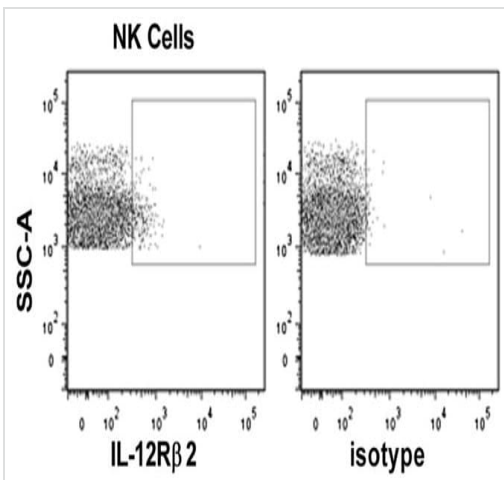


Flow Cytometry - PE/Cy5 Conjugation Kit Lightning-Link(ab102893)

Image from White, Matthew J., et al., Immunology, 142(1); doi: 10.1111/imm.12239. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/3.0/>

White, Matthew J., et al used PE/Cy5® Conjugation Kit - Lightning-Link® (ab102893) as part of examining Interleukin 12 receptor β2 (IL12Rβ2) expression relatively to CD57 expression in Peripheral blood mononuclear cells (PBMC). They used the kit to conjugate PE/Cy5® to anti-IL12Rβ2 monoclonal antibody for use in flow cytometry.

PBMC were analysed ex vivo for IL12Rβ2 expression. (a) Representative flow cytometry plots for IL12Rβ2. Frequency (b) and mean fluorescence intensity (MFI) (c) of IL12Rβ2 expression were assessed by subset. Each data point represents one donor, n = 19. Lines indicate median values. CD56dim subsets were analysed for linear trend with a repeated measures analysis of variance. ****P ≤ 0.0001.

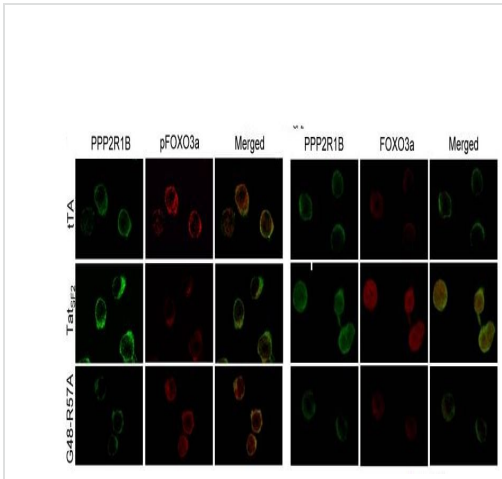


Flow Cytometry - PE/Cy5 Conjugation Kit- Lightning-Link (ab102893)

Image from Nielsen, Carolyn M., et al., J Immunol., 194(10):4657-67; doi: 10.4049/jimmunol.1403080. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/3.0/>

Nielsen, Carolyn M., et al used PE/Cy5® Conjugation Kit - Lightning-Link® (ab102893) to conjugate PE/Cy5® to anti-IL-12Rβ2 antibody for use in flow cytometry.

NK cells were analyzed for surface expression of 12Rβ2 using an mlgG1 PECy5-conjugated isotype control to set the gate.



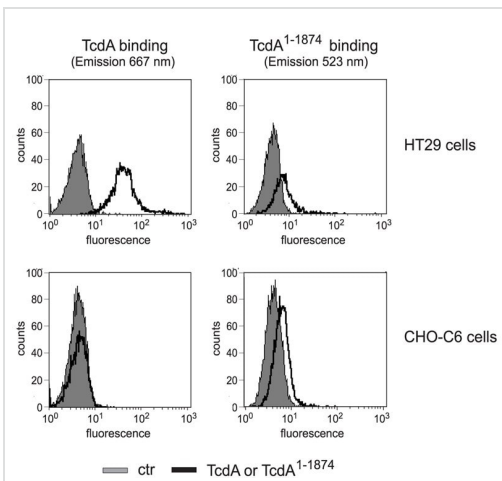
Fluorescence Microscopy - PE/Cy5 Conjugation

Kit:- Lightning-Link(ab102893)

Image from Kim, Nayoung, et al., PLoS Pathog.,6(9): e1001103; doi: 10.1371/journal.ppat.1001103. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

Kim, Nayoung, et al used PE/Cy5® Conjugation Kit - Lightning-Link® (ab102893) as part of examining apoptosis in HIV-1-infected CD4+ primary T cells. They used the kit to conjugate PE/Cy5® to anti-FOXO3a antibody for use in confocal microscopy.

Jurkat T cells expressing tTA alone, TatSF2+tTA, or TatSF2G48-R57A +tTA were stained with antibodies against PPP2R1B (first and forth columns of panels, green), pFOXO3a (second column, red), and FOXO3a (forth column, red).

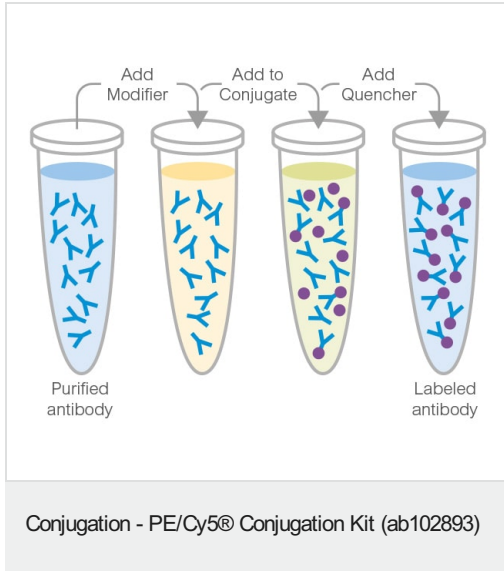


Flow Cytometry - PE/Cy; Conjugation Kit:-

Lightning-Link (ab102893)

Image from Olling, Alexandra, et al., PLoS One, 6(3):e17623. doi: 10.1371/journal.pone.0017623. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

Olling, Alexandra, et al used PE/Cy5® Conjugation Kit - Lightning-Link® (ab102893) as part of examining the role of toxins in the pathogenicity of Clostridium difficile. They used the kit to conjugate PE/Cy5® to for use in flow cytometry. Binding of fluorescent labeled TcdA-PE/Cy5 and TcdA11874-Atto488 to HT29 and CHO-C6 cells was investigated by FACS analysis. Right shift of the black curve illustrates toxin binding which was detected through fluorescence emission at 667 nm for TcdA and at 523 nm for TcdA1-1874, respectively. Due to different ratio of fluorophor and toxin, fluorescence intensity of TcdA-PE/Cy5 cannot directly be compared with TcdA1-1874-Atto488.



This illustration demonstrates a general procedure and will slightly vary dependent on the conjugate used.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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