abcam

Product datasheet

PE/Cy7® Conjugation Kit - Lightning-Link® ab102903

44 References 6 图像

概述

产品名称

产品概述

PE/Cy7®偶联试剂盒 - Lightning-Link®

PE/Cy7[®] Conjugation Kit / PE/Cy7[®] Labeling Kit ab102903 uses a simple and quick process for PE/Cy7 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/Cy7[®] using this kit:

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

The PE/Cy7[®] 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.

The excitation and emmision wavelengths for PE/Cy7® are Ex: 496nm, Em: 774nm

Learn about buffer compatibility below; for incompatible buffers and low antibody concentrations, use our rapid <u>antibody purification and concentration kits</u>. Use the <u>FAQ</u> to learn more about the technology, or about conjugating other proteins and peptides to PE/Cy7[®].

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

This product is manufactured by Expedeon, an Abcam company, and was previously called Lightning-Link $^{\circledR}$ R-PE/Cy7 Labeling Kit. 762-0005 is the same as the 60 μ g size. 762-0010 is the same as the 3 x 60 μ g size. 762-0030 is the same as the 3 x 10 μ g size. 762-0015 is the same as the 600 μ g size.

Amount and volume of antibody for conjugation to PE/Cy7[®].

Kit size	Recommended amount of antibody	Maximum antibody volume ¹
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

说明

1

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

¹ 1% BSA gives lower quality conjugates, 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.

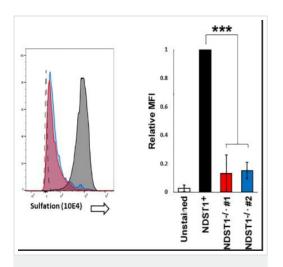
Storing and handling conjugation kits

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.

组 件	60 µg	600 µg	3 x 10 µg	3 x 60 µg
Modifier reagent	1 x 200µl	1 x 200µl	1 x 200µl	1 x 200µl
ab274150 - PE/Cy7 mix	1 x 60µg	1 x 600µg	3 x 10µg	3 x 60µg
ab274133 - Quencher reagent	1 x 200µl	1 x 200µl	1 x 200µl	1 x 200µl

图片

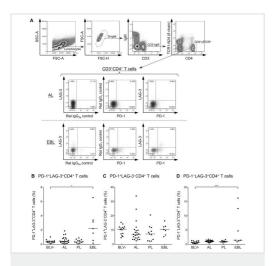


Flow Cytometry - PE/Cy7 Conjugation Kit Lightning-Link® (ab102903)

Image from Flores et al., PloS one, 15(4): e0231977; doi: 10.1371/journal.pone.0231977. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

Flores, Erica B et al. used PE/Cy7[®] Conjugation Kit - Lightning-Link[®] as part of examining Poxiviruses. They used the kit to conjugate sulfated heparin (clone F58-10E4) for use in flow cytometry.

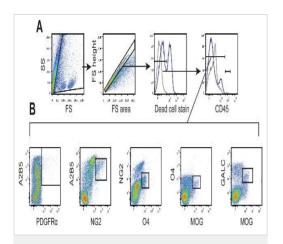
Sulfation of cell surface heparin measured via flow cytometry utilizing an antibody that recognizes the sulfated 10E4 epitope on HS chains. The mean fluorescent intensity (MFI) values were then normalized to NDST+ values to show the relative change in the NDST-/- cell lines



Flow Cytometry - PE/Cy7 Conjugation Kit;-Lightning-Link

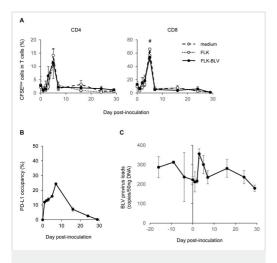
Image from Okagawa, Tomohiro et al., Vet Res., 49(1):50. doi: 10.1186/s13567-018-0543-9. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

Okagawa, Tomohiro, et al used PE/Cy7® Conjugation Kit - Lightning-Link® (ab102903) as part of examining PD-1 and LAG-3 expression. They used the kit to conjugate PE/Cy7® to monoclonal anti-lgM antibody, clone IL-A30, for use in flow cytometry. Expression of PD-1 and LAG-3 on CD4+ T cells in BLV-infected cattle. A Gating strategy and representative dot plots for expression analyses of PD-1 and LAG-3 on lgM-CD3+CD4+ $\gamma\delta$ TCR- T cells from peripheral blood of BLV-infected cattle (AL and EBL). Values in the quadrants indicate percentages of cells. Percentages of PD-1+LAG-3+CD4+ T cells (B), PD-1+LAG-3-CD4+ T cells (C), and PD-1-LAG-3+CD4+ T cells (D) in CD3+CD4+ T-cell population in peripheral blood from BLV-uninfected (BLV - ; n = 15), AL (n = 22), PL (n = 11), and EBL cattle (n = 7). Bars indicate group median percentage. Significant differences between each group were determined using a Kruskal-Wallis test, where P < 0.05 and P <



Flow Cytometry - PE/Cy7 Conjugation Kit- Lightning-Link

Image from Robinson, Andrew P., et al., PloS one; 9(9):e107649. doi: 10.1371/journal.pone.0107649. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/



Flow Cytometry - PE/Cy7® Conjugation Kit - Lightning-Link® (ab102903)

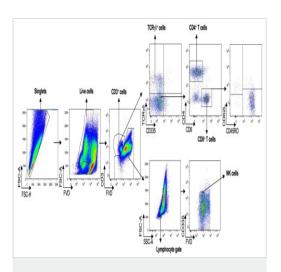
Image from Nishimori, Asami et al. PloS one vol. 12,4 e0174916. 26 Apr. 2017, doi:10.1371/journal.pone.0174916. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

Robinson, Andrew P., et al used PE/Cy7[®] Conjugation Kit - Lightning-Link[®] (ab102903) as part of characterizing oligodendroglial populations. They used the kit to conjugate PE/Cy7[®] to Mouse anti-A2B5 antibody, clone 105, for use in flow cytometry.

SJL/J mice were immunized with PLP139–151 and scored daily for clinical disease. A cohort of SJL/J mice was sacrificed, and spinal cords were analyzed by flow cytometry (n=5). (A) Cells were distinguished from debris by forward and side scatter then singlet cells were gated. Live cells were gated by dead cell exclusion, and CNS resident cells were identified as CD45– or CD45low. (B) Oligodendroglial cells were defined by double positive staining: A2B5+PDGFRα+ early OPCs, A2B5+NG2+ intermediate OPCs, NG2+O4+ late OPCs, O4+MOG+ pre-myelinating oligodendrocytes, and GALC+MOG+ mature oligodendrocytes.

Nishimori, Asami et al used PE/Cy7[®] Conjugation Kit - Lightning-Link[®] (ab102903) as part of examining bovine leukemia virus infection. They used the kit to conjugate PE/Cy7[®] to anti-bovine lgM antibody for use in flow cytometry.

A BLV-infected cow (#368, Holstein, female, 538 kg, 31 months old) was inoculated with 530 mg (1 mg/kg) of the purified 4G12 intravenously. (A) The proliferation of CD4+ and CD8+ T cells against BLV antigen. Peripheral blood mononuclear cells (PBMCs) isolated from the cow which was inoculated with 4G12 were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) and cultured without stimulation (medium) or with the supernatant of FLK or FLK-BLV cells for 6 days. After the cultivation, the proliferation of T cells was immediately analyzed by flow cytometry. A P-value less than 0.05 was considered statistically significant. #, P < 0.05 (FLK-BLV, versus day 0; one-way ANOVA followed by Dunnett's test). (B) Changes in PD-L1 occupancy on circulating IgM+ B cells calculated by the binding of 4G12 to bovine PD-L1. The occupancy was estimated as the percentage of the in vivo PD-L1 binding occurred at the total available binding sites. (C) Changes in BLV provirus loads in the cow inoculated with 4G12; the y-axis shows the number of BLV copies included in 50-ng DNA extracts of PBMCs. Data are means ± SEM of at least three replicate experiments.



ab102903 PE-Cy7® conjugation kit used with a mouse anti-bovine CD45RO antibody

Image from Oliveira BMet al., Scientific reports., 9 (1) 3413. Fig 1.; doi: 10.1038/s41598-019-39938-0. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/.

Add to Add to Quencher Quencher Purified antibody

PE/Cy7 Kit

Oliveira BM et al. used ab102903 PE-Cy7[®] conjugation kit with a mouse anti-bovine CD45RO antibody and <u>ab102859</u> APC-Cy7[®] conjugation kit with a mouse anti-Bovine CD62L antibody. This enabled them to run their desired multicolor flow cytometry panel.

Data shows flow cytometry gating strategy used to define $\gamma\delta$ T cells (TCR $\gamma\delta$ +CD3+CD335-), CD4+ T cells (CD4+CD3+TCR $\gamma\delta$ -CD335-), CD8+ T cells (CD8+CD3+TCR $\gamma\delta$ -CD335-) and NK cells (CD335+CD3-) in the stromal vascular fraction (SVF) of mesenteric and subcutaneous bovine adipose tissue (MAT and SAT, respectively) and in peripheral blood leukocytes. Dead cells were excluded with Fixable Viability Dye (FVD), lymphocytes were gated based on SSC-A versus FSC-A and singlets were selected from the FSC-A versus FSC-H dot plot.

The flow cytometry gating strategy used to define CD45RO+ and CD62L+ T cell subpopulations is also shown in CD8+

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

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