abcam

Product datasheet

# APC/Cy7® Conjugation Kit - Lightning-Link® ab102859

19 References 8 Images

## Overview

**Product name** APC/Cy7® Conjugation Kit - Lightning-Link®

**Product overview** APC/Cy7® Conjugation Kit / APC/Cy7® Labeling Kit ab102859 uses a simple and quick process for APC/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 APC/Cy7® using this kit:
- add modifier to antibody and incubate for 3 hrs
- add quencher and incubate for 30 mins
The APC/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 APC/Cy7® are Ex: 650nm, Em: 774nm.

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 APC/Cy7®.

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® APC/Cy7 Labeling Kit. 765-0015 is the same as the 1 mg size. 765-0010 is the same as the 3 x 100 ug size. 765-0030 is the same as the 3 x 10 ug size. 765-0005 is the same as the 100 µg size.

**Amount and volume of antibody for conjugation to APC/Cy7®.**

| *Kit size* | *Recommended maximum amount of antibody* | *Maximum antibody volume[1]* |
|---|---|---|
| 3 x 10 µg | 3 x 10 µg | 3 x 10 µL |
| 100 µg | 1 x 100 µg | 1 x 100 µL |
| 3 x 100 µg | 3 x 100 µg | 3 x 100 µL |

| | | |
|---|---|---|
| 1 mg | 1 x 1 mg | 1 x 1 mL |

[1] Ideal 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.

### 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[3] | 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.

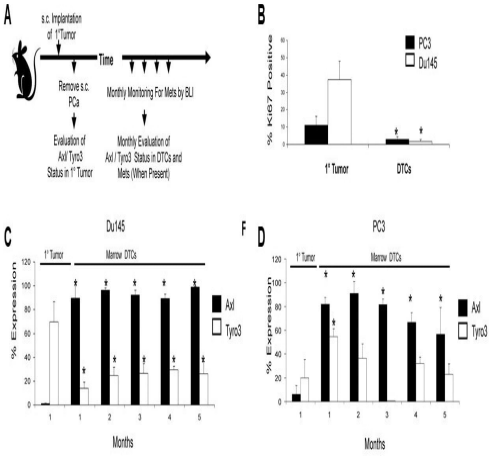
## Properties

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

| Components | 1 mg | 100 µg | 3 x 10 µg | 3 x 100 µg |
|---|---|---|---|---|
| ab274159 - APC-Cy7 Mix | 1 x 1mg | 1 x 100µg | 3 x 10µg | 3 x 100µg |
| Modifier reagent | 1 x 200µl | 1 x 200µl | 1 x 200µl | 1 x 200µl |

| Components | 1 mg | 100 µg | 3 x 10 µg | 3 x 100 µg |
|---|---|---|---|---|
| ab274133 - Quencher reagent | 1 x 200µl | 1 x 200µl | 1 x 200µl | 1 x 200µl |

## Images

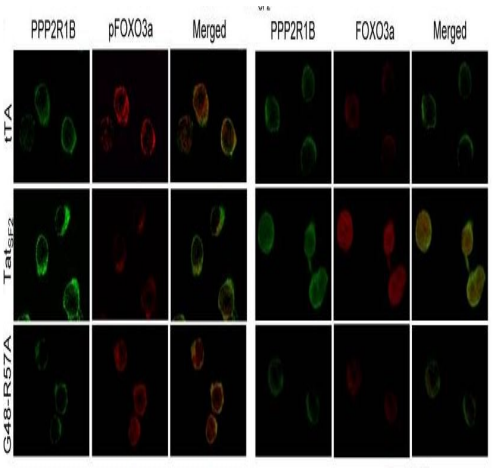



Flow Cytometry - APC/Cy7; Conjugation Kit-Lightning-Link (ab102859)



Taichman, Russell S., et al used APC/Cy7® Conjugation Kit - Lightning-Link® (ab102859) as part of examining Axl and Tyro3 expression during experimental prostate cancer (PCa) progression. They used the kit to conjugate APC/Cy7® 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.

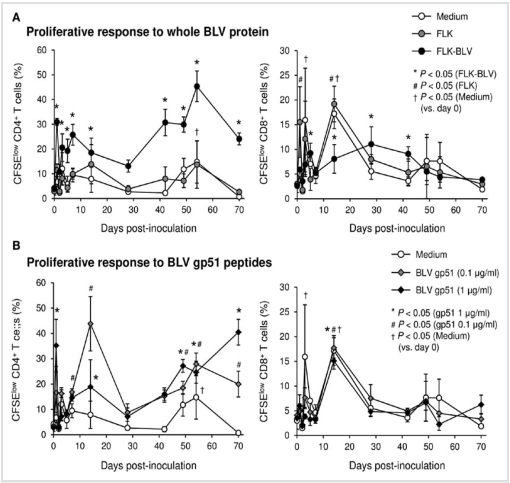



Fluorescence Microscopy - APC/Cy7 Conjugation Kit- Lightning-Link(ab102859)



Kim, Nayoung, et al used APC/Cy7® Conjugation Kit - Lightning-Link® (ab102859) as part of examining apoptosis in HIV-1-infected CD4+ primary T cells. They used the kit to conjugate APC/Cy7® to anti-PPP2R1B 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).

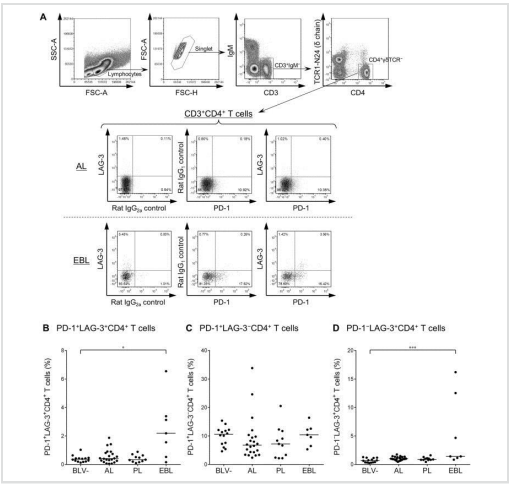



Flow Cytometry - APC/Cy7 Conjugation Kit-Lightning-Link

Image from Okagawa, Tomohiro, et al., Front Immunol., 8:650, doi: 10.3389/fimmu.2017.00650. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

Okagawa, Tomohiro, et al used APC/Cy7® Conjugation Kit - Lightning-Link® (ab102859) as part of examining the effect on proliferation of bovine leukemia virus (BLV)-specific T cells of the administration of Boch5D2. They used the kit to conjugate APC/Cy7® to monoclonal anti-TCR1-N24 (δ chain) antibody, clone GB21A, for use in flow cytometry.

T-cell proliferation specific for BLV antigen stimulation. Carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled peripheral blood mononuclear cells were cultured in triplicate with fetal lamb kidney (FLK)-BLV antigen, control FLK antigen (A), or gp51 peptides (0.1 and 1 μg/ml) (B) for 6 days. The percentage of CFSElow cells in CD4+ and CD8+γδTCR− T cells was measured by flow cytometry. CFSElow cells represent cells proliferated during cultivation. Each dot represents the mean of three independent experiments. Significant differences were determined by Dunnett's multiple-comparison test across the time points. *,#,†P < 0.05 versus 0 dpi in each stimulation.

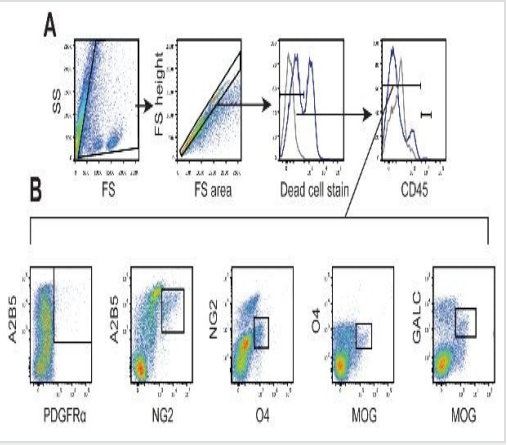



Flow Cytometry - APC/Cy7 Conjugation Kit-Lightning-Link (ab102859)

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 APC/Cy7® Conjugation Kit - Lightning-Link® (ab102859) as part of examining PD-1 and LAG-3 expression. They used the kit to conjugate APC/Cy7® to monoclonal anti-TCR1-N24 (δ chain) antibody, clone GB21A, 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 IgM−CD3+CD4+γδTCR− 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 < 0.001, indicated by asterisks (* and ***, respectively).

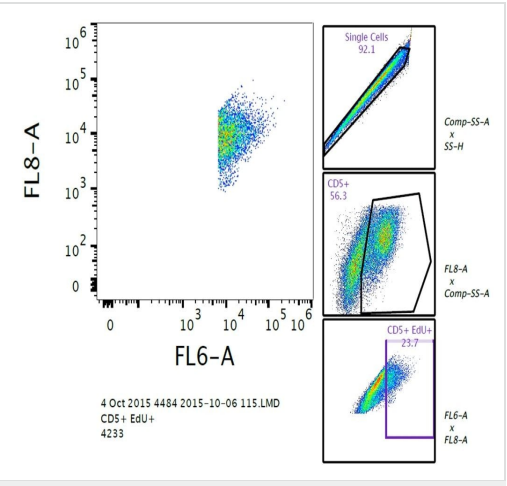



Flow Cytometry - APC/Cy7 Conjugation Kit - Lightning-Link



Robinson, Andrew P., et al used APC/Cy7® Conjugation Kit - Lightning-Link® (ab102859) as part of characterizing oligodendroglial populations. They used the kit to conjugate APC/Cy7® to Mouse anti-MOG antibody, clone 8-18C5, 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.

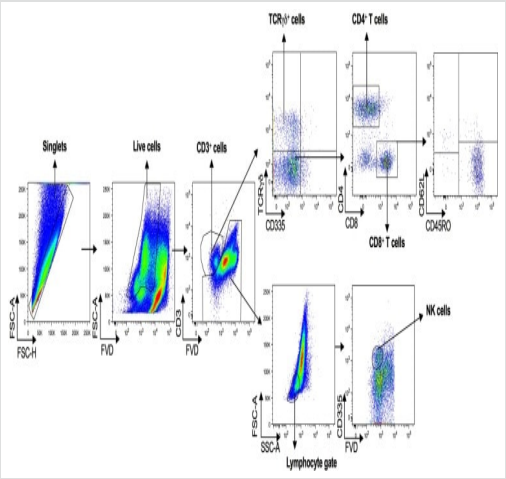



Flow Cytometry - APC/Cy7; Conjugation Kit ;- Lightning-Link



Kopanke, Jennifer H., et al used APC/Cy7® Conjugation Kit - Lightning-Link® (ab102859) to identify CD5+/5-ethynyl-2′-deoxyuridine (EdU)+ cells. They used the kit to conjugate APC/Cy7® to Mouse Anti-Feline CD5 antibody for use in cell proliferation assay.

Gating tree used to identify CD5+/5-ethynyl-2′-deoxyuridine (EdU)+ cells. Single cells were identified by plotting side scatter area by side scatter height. Cells aligning linearly were counted as single cells. CD5+ cells were identified from single cells by plotting FL8 (the channel recognizing APC-Cy7) by side scatter area. Finally, CD5+ EdU+ cells were identified from CD5+ cells by plotting FL6 (the channel that detects APC) by FL8.



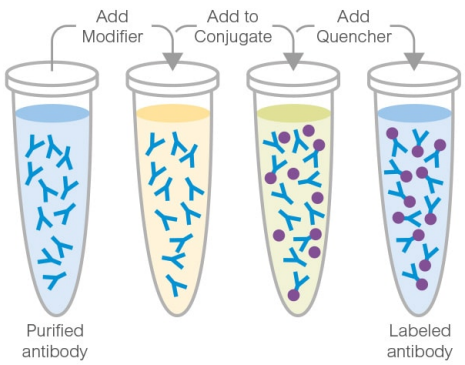

ab102859 APC-Cy7® conjugation kit used with a mouse anti-Bovine CD62L antibody

Image from Oliveira BM et 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/.

Oliveira BM et al. used **ab102903** PE-Cy7® conjugation kit with a mouse anti-bovine CD45RO antibody and ab102859 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 γδ T cells (TCRγδ+CD3+CD335−), CD4+ T cells (CD4+CD3+TCRγδ−CD335−), CD8+ T cells (CD8+CD3+TCRγδ−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+ T cells.




conjugation

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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