

# APC/Cy5.5® Conjugation Kit - Lightning-Link® ab102855

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### Overview

**Product name** APC/Cy5.5® Conjugation Kit - Lightning-Link®

**Product overview** APC/Cy5.5® Conjugation Kit / APC/Cy5.5® Labeling Kit ab102855 uses a simple and quick process for APC/Cy5.5 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/Cy5.5® using this kit:

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

The APC/Cy5.5 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 APC/Cy5.5®.

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/Cy5.5 Labeling Kit. 764-0015 is the same as the 1 mg size. 764-0010 is the same as the 3 x 100 µg size. 764-0030 is the same as the 3 x 10 µg size. 764-0005 is the same as the 100 µg size.

#### Amount and volume of antibody for conjugation to APC/Cy5.5®.

<i>Kit size</i>	<i>Recommended maximum amount of antibody</i>	<i>Maximum antibody volume<sup>1</sup></i>
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
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<sup>1</sup> 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 <sup>1</sup>	0.1% BSA	50% glycerol
0.1% sodium azide	PBS	Potassium phosphate
Sodium chloride	HEPES	Sucrose
Sodium citrate	EDTA	Trehalose

<sup>1</sup> 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<sup>®</sup> 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

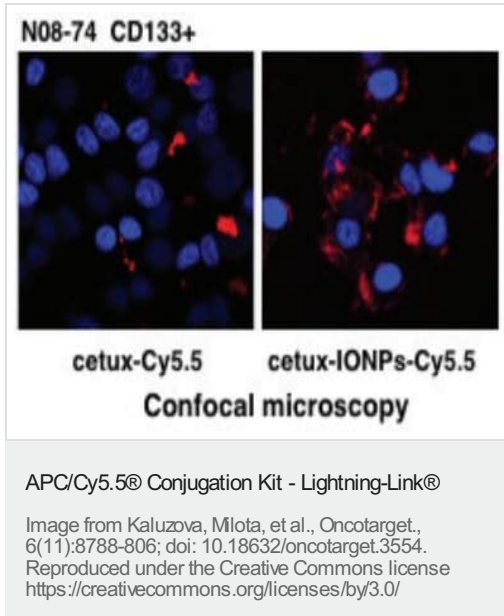
### Storage instructions

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

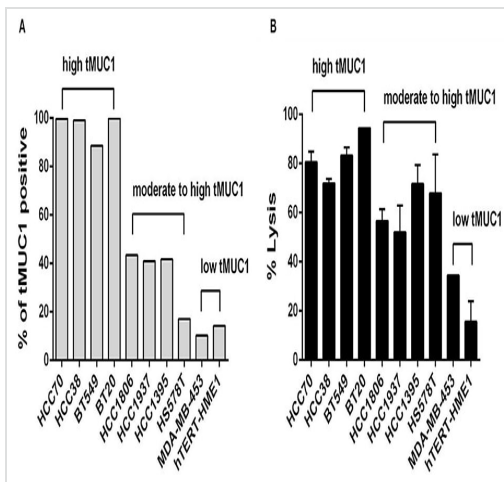
Components	1 mg	100 µg	3 x 10 µg	3 x 100 µg
ab274156 - APC-CY5.5 Mix	1 x 1mg	1 x 100µg	3 x 10µg	3 x 100µg
Modifier reagent	1 x 200µl	1 x 200µl	1 x 200µl	1 x 200µl

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

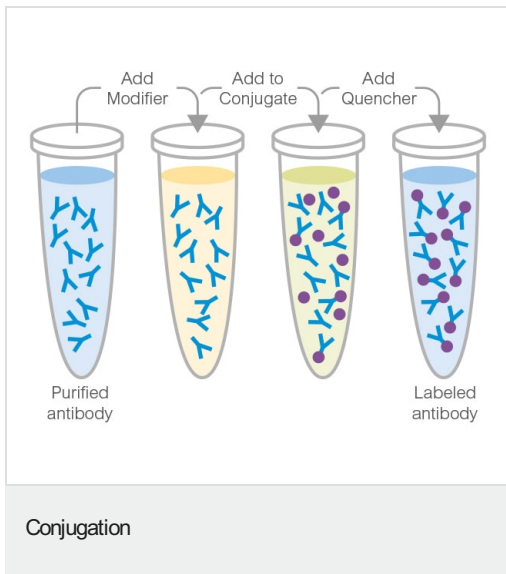
## Images



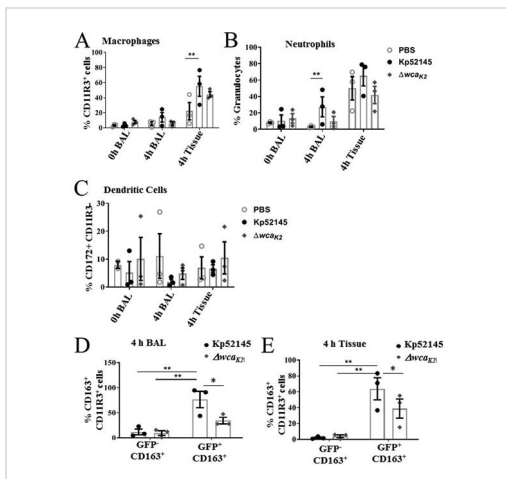
Kaluzova, Milota, et al used APC/Cy5.5® Conjugation Kit - Lightning-Link® (ab102855) as part of a molecular profiling and characterization of human GSCs. They used the kit to conjugate APC/Cy5.5® to cetuximab-IONPs for use in immunocytochemistry/immunofluorescence (ICC/IF). Confocal microscopy of cetuximab-Cy5.5 and cetuximab-IONPs-Cy5.5 internalized by CD133-positive Glioblastoma stem-like cells (GSCs) from Glioblastoma neurosphere N08-74. After 4 h treatment, GSCs were allowed to attach to culture slides, fixed, and imaged using Zeiss LSM 510 Meta Confocal microscope. Cy5.5, pseudo-colored red; DAPI, pseudo-colored blue (maximum intensity projection, magnification 100x).



Zhou, Ru, et al used APC/Cy5.5® Conjugation Kit - Lightning-Link® (ab102855) as part of examining the MUC28z CAR T cells lysis of TNBC tumor cells in vitro in an antigen-dependent manner. They used the kit to conjugate APC/Cy5.5® to monoclonal anti-tMUC1 antibody, clone TAB004, for use in flow cytometry. (A) Percentage of cells expressing tMUC1, determined by TAB004-APC/Cy5.5 staining and flow cytometry. A panel of nine TNBC cell lines and one "normal" mammary epithelial cell line hTERT-HME1 is shown. (B) Percentage of TNBC tumor cell lysis by MUC28z CAR T cells. Cells were co-cultured at E:T ratio of 5:1 for 3 days. The lysis of tumor cells was determined by MTT assay. Data are presented as the mean  $\pm$  SEM. The relationship between tMUC1 positivity in TNBCs and tumor lysis by MUC28z CAR T cells was analyzed by a non-parametric Spearman correlation, with  $r = 0.8909$  which was highly significant ( $P = 0.0011$ ) and indicated a positive association between tMUC1 level and tumor lysis.



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



#### Flow Cytometry - APC/Cy5.5 Conjugation Kit Lightning-Link (ab102855)

Image from Dumigan, Amy, et al., *MBio.*, 10(6): e02802-19; doi: 10.1128/mBio.02802-19. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

Dumigan, Amy, et al used APC/Cy5.5® Conjugation Kit - Lightning-Link® (ab102855) as part of examining innate cell recruitment in *K. pneumoniae*-infected porcine EVLP model. They used the kit to conjugate APC/Cy5.5® to monoclonal anti-CD163 antibody, clone 2A10, for use in flow cytometry.

(A) CD11R3<sup>+</sup> macrophages at baseline (0 h) and endpoint (4 h posttreatment) in BAL samples and tissue from caudal lobes mock infected (PBS) or infected with Kp52145 and strain 52145-ΔwcaK2. (B) Granulocytes at baseline (0 h) and endpoint (4 h posttreatment) in BAL samples and tissue from caudal lobes mock infected (PBS) or infected with Kp52145 and strain 52145 wcaK2. (C) CD172<sup>+</sup> dendritic cells at baseline (0 h) and endpoint (4 h posttreatment) in BAL samples and tissue from caudal lobes mock infected (PBS) or infected with Kp52145 and strain 52145- wcaK2. (D and E) Percentages of CD11R3<sup>+</sup> macrophages positive for CD163 expression associated (GFP<sup>+</sup>) or not (GFP<sup>-</sup>) with Kp52145 and strain 52145- wcaK2 harboring plasmid pFPV25.1 Cm in BAL fluid (D) and tissue (E). In all panels, values are represented as means ±SEM from three independent experiments. \*\*, P < 0.001; \*, P < 0.05 (determined by unpaired t test).

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