

PE/Cy5.5[®] Conjugation Kit - Lightning-Link[®] ab102899

[9 References](#) [3 Images](#)

Overview

Product name

PE/Cy5.5[®] Conjugation Kit - Lightning-Link[®]

Product overview

PE/Cy5.5[®] Conjugation Kit / PE/CY5.5[®] Labeling Kit **ab102871** uses a simple and quick process for PE/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 PE/Cy5.5[®] using this kit:

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

The 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/Cy5.5[®].

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[®] R-PE/Cy5.5 Labeling Kit. 761-0005 is the same as the 60 µg size. 761-0010 is the same as the 3 x 60 µg size. 761-0030 is the same as the 3 x 10 µg size. 761-0015 is the same as the 600 µg size.

Amount and volume of antibody for conjugation to PE/Cy5.5[®].

<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 1 mg/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.

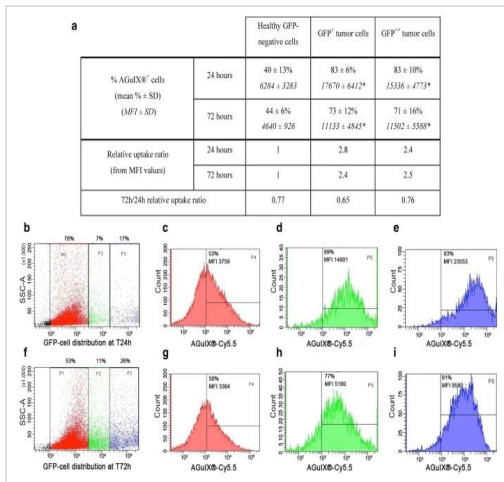
Properties

Storage instructions

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

Components	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
ab274147 - PE/Cy5.5 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

Images



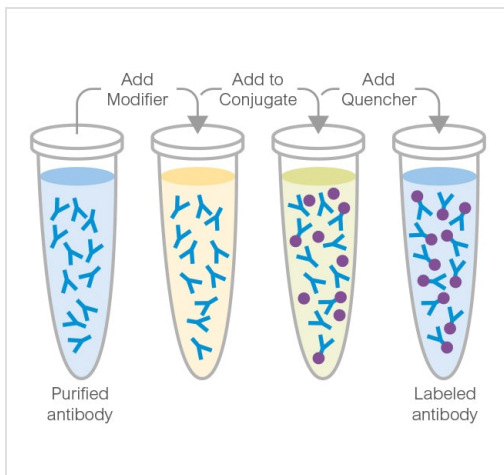
Flow Cytometry - PE/Cy5.5 Conjugation Kit

Lightning-Link; (ab102899)

Image from Sancey, Lucie, et al., *J. nanobiotechnology*, 18(1):129; doi: 10.1186/s12951-020-00683-6. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

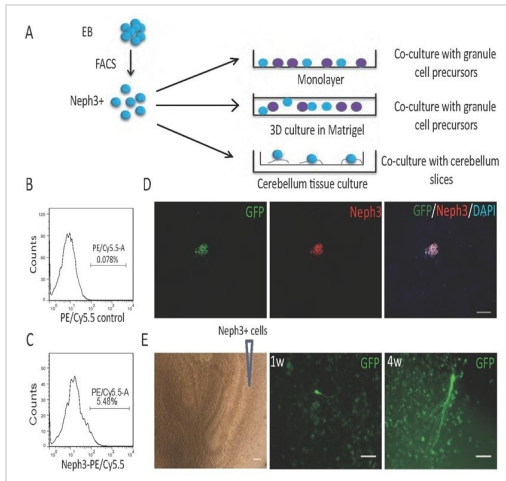
Sancey, Lucie, et al used PE/Cy5.5® Conjugation Kit - Lightning-Link® (ab102899) as part of examining AGuIX NP uptake measured by FCM as a function of time in the 3 OncoCilAir™ subpopulations. They used the kit to conjugate PE/Cy5.5® to IgG isotype control antibody for use in flow cytometry.

a Summary of AGuIX® uptake with the percentage of AGuIX⁺ cells and their corresponding MFI values presented after 24 and 72 h of NP exposure (n=6/conditions, from 3 distinct batches of cell culture with and without mucus). The relative uptake ratios were calculated from Eq. 3. Statistical analysis was performed using the Mann-Whitney test (*p 0.05 compared to GFP-negative cells). The results b-i were obtained from samples of the 2nd series of cell cultures, presenting a fast tumor growth and an intense AGuIX® uptake at T24h. FACS plots of AGuIX® uptake (% of AGuIX⁺-cells and geometric mean MFI) according to the 3 gates (b, f) that discriminate GFP-negative (red channel; c, g), GFP+ (green channel; d, h) and GFP++ (blue channel; e, i) tumor cells at 24 h (b-e) and 72 h (f-i)



Conjugation

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



Flow Cytometry - PE/Cy5.5 Conjugation Kit-
Lightning-Link(ab102899)

Image from Wang, Shuyan, et al., Scientific reports, 5:9232; doi: 10.1038/srep09232. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

Wang, Shuyan, et al used PE/Cy5.5® Conjugation Kit - Lightning-Link® (ab102899) as part of examining Neph3-positive cells co-cultured with rat cerebellar slices. They used the kit to conjugate PE/Cy5.5® to anti-Neph3 monoclonal antibody for use in flow cytometry.

(A) Schematic representation of sorting and co-culture procedures. (B, C) FACS analysis of neural rosette cells on Day 20 of differentiation. (D) Immunofluorescence staining to confirm that the sorted cells were Neph3/GFP-double positive. Bar = 100 µm. (E) The sorted cells were co-cultured with cerebellum slices and examined at 1 week and 4 weeks. Bar = 100 µm.

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