

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

For simple and quick conjugation of IgG antibodies with PE/Cy5.5®.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab102899
(use www.abcam.cn/ab102899 for China, or www.abcam.co.jp/ab102899 for Japan)

Introduction:

PE/Cy5.5® Conjugation Kit - Lightning-Link® (ab102899) provides a simple and rapid procedure to covalent labeling of antibodies with PE/Cy5.5® by targeting primary amine groups (e.g. lysines). It takes only 30 seconds hands-on time and conjugates are ready to use in less than 3 hours 30 minutes without loss of antibodies.

This protocol is optimized for conjugation of IgG antibodies, for other proteins and biomolecules containing amine groups, please consult our guide.

<https://www.abcam.com/NonIggConjugation>

The antibody to be labeled should be purified, in an appropriate buffer for conjugation and at a suitable concentration conjugation (as described in buffer considerations).

Abcam offers the widest range of carrier-free antibodies for antibody labeling. Our conjugation-ready antibodies are affinity purified, manufactured in a buffer free from BSA, sodium azide and glycerol, and supplied at an optimal concentration of 1 mg/ml.

<https://www.abcam.com/carrier-free>

If the antibody of choice is supplied in a buffer not compatible with conjugation (as described in buffer considerations), we offer a dedicated range of antibody purification and concentration kits.

<https://www.abcam.com/PurificationKits>

The kit comes in 4 pack sizes:

- 3 x 10 µg containing 3 reactions (each for 5 - 10 µg)
- 60 µg containing 3 reactions (for 30 - 60 µg)
- 3 x 60 µg containing 3 reactions (each for 30 - 60 µg)
- 600 µg containing 1 reaction (for 300 - 600 µg)

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

Materials Supplied:

Item	3 x 10 µg	60 µg	3 x 60 µg	600 µg	Storage temperature
PE/Cy5.5® mix	3 vials	1 vial	3 vials	1 vial	-20°C
Modifier reagent	1 vial	1 vial	1 vial	1 vial	-20°C
Quencher reagent	1 vial	1 vial	1 vial	1 vial	-20°C

Storage and Stability: Store kit at -20°C immediately upon receipt.

Buffer considerations:

Recommended pre-conjugation buffer components and conditions:

Buffer Components & Conditions	
Purified antibody	Yes
Antibody in ascites fluid, serum, hybridoma or tissue culture media	No
Antibody concentration	0.5-1 mg/mL
pH	6.5-8.5
Amine free buffer (e.g. MES, MOPS, HEPES, PBS)	Yes
Non-buffering salts (e.g. sodium chloride)	Yes
BSA ¹	<0.1%
Sodium Azide ¹	<0.1%
Chelating agents (e.g. EDTA)	Yes
Glycerol	<50%
Sugars	Yes
Gelatin ¹	<0.1%
Tris	<50mM
Glycine	No
Thiomersal / Thimerosal	No
Merthiolate	No
Proclin	No
Borate buffer	Yes
Nucleophilic components (Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)	No

ΔNote: ¹ Individually, these components and conditions should not affect the reaction. In combination however, the reaction may be affected.

Recommended starting amount and volume of antibody:

Vial Size	Lowest Amount of Antibody	Recommended and Maximum Amount of Antibody	Fixed Volume of Antibody
10 µg	Down to 5 µg at 0.5 mg/mL	Optimally and maximum 10 µg at 1 mg/mL	Fixed 10 µL
60 µg	Down to 30 µg at 0.5 mg/mL	Optimally and maximum 60 µg at 1 mg/mL	Fixed 60 µL
600 µg	Down to 300 µg at 0.5 mg/mL	Optimally and maximum 600 µg at 1 mg/mL	Fixed 600 µL

Δ Note: It is important to use the indicated fixed volumes. Modifying the reaction volume may result in poor quality conjugates.

Δ Note: Antibodies can be diluted using either milli-Q water or PBS.

Δ Note: Optimal conjugates are normally generated using the recommended amount of antibody, although using different amounts of antibody within the range provided will still generate quality conjugates.

Δ Note: Adding less than the recommended amount of antibody may result in unbound label post conjugation. This excess label will be deactivated by the quencher and removed during the first wash step of any application.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.

1. Add 1 µL of Modifier reagent to each 10 µL of antibody to be labeled and mix gently.
2. Remove cap from vial of PE/Cy5.5® Conjugation Mix and pipette the antibody sample (with added Modifier reagent) directly onto the lyophilized material. Resuspend gently by withdrawing and re-dispensing the liquid once or twice using a pipette.
3. Replace cap on the vial and leave standing for 3 hours in the dark at room temperature (20-25°C). Longer incubation times, such as overnight, have no negative effect on the conjugation.
4. After incubating for 3 hours (or more), add 1 µL of Quencher reagent for every 10 µL of antibody used and mix gently. The conjugate can be used after 30 minutes. The conjugates do not require purification.

Conjugate Storage

Typically, the antibody conjugate can be stored at 4°C for up to 3 months. For longer storage the conjugate can be stored at -20°C with a cryoprotectant such as 50% glycerol. The best storage conditions for any particular conjugate must be determined by experimentation and depend on the antibody stability and best storage conditions. The antibody conjugate should always be stored in the dark.

Technical Support

Copyright © 2020 Abcam. All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)



To get Chinese protocol, please scan QR code via WeChat or visit Protocols section at www.abcam.cn/ab102899.