

## ab269897 – Atto 565 Conjugation Kit - Lightning-Link®

For simple and quick conjugation of IgG antibodies with Atto 565.  
For research use only - not intended for diagnostic use.

**For overview, typical data and additional information please visit:** [www.abcam.com/ab269897](http://www.abcam.com/ab269897)  
(use [www.abcam.cn/ab269897](http://www.abcam.cn/ab269897) for China, or [www.abcam.co.jp/ab269897](http://www.abcam.co.jp/ab269897) for Japan)

### Introduction:

Atto 565 Conjugation Kit - Lightning-Link® (ab269897) provides a simple and rapid procedure to covalent labeling of antibodies with Atto 565 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 20 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 3 pack sizes:

- 3 x 10 µg containing 3 reactions (each for 5 - 20 µg)
- 100 µg containing 1 reaction (for 50 - 200 µg)
- 3 x 100 µg containing 3 reactions (each for 50 - 200 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 Atto 565 Labeling Kit. 351-0030 is the same as the 3 x 10 µg size. 351-0005 is the same as the 100 µg size. 351-0010 is the same as the 3 x 100 µg size.

### Materials Supplied:

Item	3 x 10 µg	100 µg	3 x 100 µg	Storage temperature
Atto 565 mix	3 vials	1 vial	3 vials	-20°C
Modifier reagent	1 vial	1 vial	1 vial	-20°C
Quencher reagent	1 vial	1 vial	1 vial	-20°C

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

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.

### 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	0.5-2 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 <sup>1</sup>	<0.1%
Sodium Azide <sup>1</sup>	<0.1%
Chelating agents (e.g. EDTA)	Yes
Glycerol	<50%
Sugars	Yes
Gelatin <sup>1</sup>	<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 ethanalamine and thiols e.g. mercaptoethanol or DTT)	No

**ΔNote:** <sup>1</sup> 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 Amount of Antibody	Maximum Amount of Antibody	Fixed Volume of Antibody
10 µg	Down to 5 µg at 0.5 mg/mL	Optimally 10 µg at 1 mg/mL	Up to 20 µg at 2 mg/mL	Fixed 10 µL
100 µg	Down to 50 µg at 0.5 mg/mL	Optimally 100 µg at 1 mg/mL	Up to 200 µg at 2 mg/mL	Fixed 100 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 Atto 565 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 15 minutes 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 15 minutes (or more), add 1 µL of Quencher reagent for every 10 µL of antibody used and mix gently. The conjugate can be used after 5 minutes. The conjugates do not require purification.

#### Conjugate Storage

Typically, the antibody conjugate can be stored at 4°C for up to 18 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

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[www.abcam.co.jp/contactus](http://www.abcam.co.jp/contactus) (Japan)



To get Chinese protocol, please scan QR code via WeChat or visit Protocols section at [www.abcam.cn/ab269897](http://www.abcam.cn/ab269897).