# abcam

## Product datasheet

## DyLight® 650 Conjugation Kit (Fast) - Lightning-Link® ab201803

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## 概述

产品名称

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DyLight® 650偶联试剂盒(Fast) - Lightning-Link®

DyLight<sup>®</sup> 650 Conjugation Kit / DyLight<sup>®</sup> 650 Labeling Kit ab201803 uses a simple and quick process for DyLight 650 labeling / conjugation of antibodies. It can also be used to conjugate other proteins or peptides. Learn about our **antibody labeling kits and their advantages**.

This product is manufactured by Expedeon, an Abcam company. See the table below to match Abcam kit size against Expedeon product code.

To conjugate an antibody to DyLight® 650 using this kit:

- add modifier to antibody and incubate for 15 mins
- add quencher and incubate for 5 mins

The DyLight 650 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 DyLight<sup>®</sup> 650.

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 $^{\circledR}$  Rapid DyLight $^{\circledR}$  650 Labeling Kit. 326-0015 is the same as the 1 mg size. 326-0010 is the same as the 3 x 100 ug size. 326-0030 is the same as the 3 x 10 ug size. 326-0005 is the same as the 100 ug size.

## Amount and volume of antibody for conjugation to Dylight® 650

Kit size	Recommended amount of antibody <sup>1</sup>	Maximum amount of antibody	Maximum antibody volume <sup>2</sup>		
3 x 10 µg	3 x 10 µg	3 x 20 μg	3 x 10 µL		
100 µg	1 x 100 µg	1 x 200 μg	1 x 100 μL		

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3 x 100 µg	3 x 100 μg	3 x 200 µg	3 x 100 μL	
1 mg	1 x 1 mg	1 x 2 mg	1 x 1 mL	

<sup>&</sup>lt;sup>1</sup> Using the maximum amount of antibody may result in less labelling per antibody.

## **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 <sup>2</sup>	50% glycerol
0.1% sodium azide	PBS	Potassium phosphate
Sodium chloride	HEPES	Sucrose
Sodium citrate	EDTA	Trehalose

<sup>&</sup>lt;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® 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.

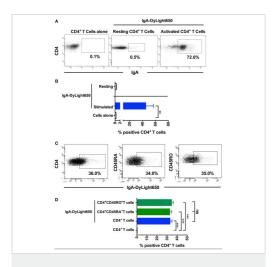
DyLight® is a trademark of Thermo Fisher Scientific Inc. and its subsidiaries.

<sup>&</sup>lt;sup>2</sup> Ideal antibody concentration is 1mg/ml. 0.5 - 1 mg/ml can be used if the maximum antibody volume is not exceeded. Antibodies > 2 mg/ml or < 0.5 mg/ml should be diluted /concentrated.

<sup>&</sup>lt;sup>2</sup> BSA can also interfere with the use of the conjugated antibody in tissue staining.

组 <b>件</b>	1 mg	100 µg	3 x 10 µg	3 x 100 μg
ab274025 - Dylight® 650 Conjugation Mix	1 x 1mg	1 x 100µg	3 x 10µg	3 x 100µg
ab273994 - Modifier reagent	1 x 200µl	1 x 200µl	1 x 200µl	1 x 200µl
ab273995 - Quencher reagent	1 x 200µl	1 x 200µl	1 x 200µl	1 x 200µl

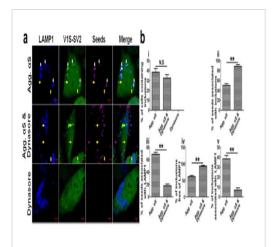
## 图片



Flow Cytometry - DyLight<;650 Conjugation Kit (Fast)- Lightning-Link (ab201803)

Image from Saha, Chaitrali, et al., Front Immunol., 8:275. doi: 10.3389/fimmu.2017.00275. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

Saha, Chaitrali, et al used DyLight<sup>®</sup> 650 Conjugation Kit (Fast) - Lightning-Link<sup>®</sup> (ab201803) as part of examining the ability of monomeric lgA (mlgA) isolated from pooled plasma of healthy donors to modulate human Th17 cells. They used the kit to conjugate DyLight<sup>®</sup> 650 to lgA and lgG for use in flow cytometry. Monomeric lgA (mlgA) binds to CD4+ T cells. (A,B) Representative dot plots and percentage (mean  $\pm$  SEM, n = 3–9 donors) of binding of DyLight650-conjugated mlgA to CD4+ T cells. Statistical significance as determined by two-tailed Student's t-test is indicated (\*\*P < 0.01). (C,D) Representative dot plots and percentage (mean  $\pm$  SEM, n = 4) of binding of DyLight650-conjugated mlgA to CD4+ T cells, CD4+CD45RA+ naïve T cells, and CD4+CD45RO+ memory T cells. Statistical significance as determined by one-way ANOVA is indicated (\*\*P < 0.01; \*\*\*P < 0.001; ns, not significant).



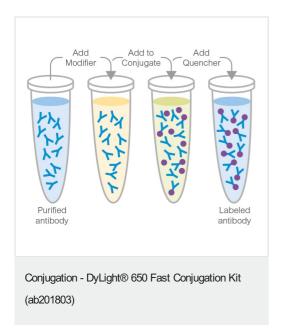
## Fluorescence Microscopy - DyLight Lightning-Link

Image from Jiang, Peizhou, et al., Scientific reports 7.1 (2017): 1-13. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

Jiang, Peizhou, et al used DyLight<sup>®</sup> 650 Conjugation Kit - Lightning-Link<sup>®</sup> (ab201803) as part of examining  $\alpha$ -synuclein aggregates. They used the kit to conjugate DyLight<sup>®</sup> 650 to  $\alpha$ -synuclein protein aggregates for use in confocal microscopy.

Membrane penetration- and endocytosis-mediated  $\alpha S$  seeding contribute to formation of endo-lysosome-free and endo-lysosome-associated  $\alpha S$  inclusions. (a) H4/V1S-SV2/LAMP1-eCFP cells were respectively treated with DyLight® 650 labeled  $\alpha S$  seeds (Agg.  $\alpha S$ ), Dynasore, and Dynasore & Agg.  $\alpha S$  for 2 days followed by imaging under confocal microscopy. The concentration for Agg.  $\alpha S$  is 0.5  $\mu g$ / ml, and Dynasore 20  $\mu M$ . Yellow and white arrows denote  $\alpha S$  seeds and induced LAMP1-free (yellow), LAMP1-positive (white)  $\alpha S$  inclusion, respectively. Scale bar: 5  $\mu m$ . (b) Bar graphs show the comparisons among Agg.  $\alpha S$ , Dynasore, and Dynasore & Agg.  $\alpha S$  treated cells in the ratio of cells with inclusions to total cells, inclusions to total seeds, LAMP1-positive seeds to total seeds, LAMP1-negative and LAMP1-positive inclusions to

total inclusions, respectively. Error bars represent standard error of the mean (N.S = non-significant; \*\*p < 0.01, comparing subsets linked by line, n = 3).



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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