# abcam

# Product datasheet

# Alkaline phosphatase Conjugation Kit - Lightning-Link® ab102850

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#### 概述

产品名称

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Alkaline phosphatase偶联试剂盒 - Lightning-Link®

An alternative Alkaline Phosphatase Conjugation Kit (Animal Free) - Lightning-Link® (ab269901) is now in stock. The alkaline phosphatase enzyme used in this kit are produced recombinantly (in vitro) for high batch-to-batch consistency and long term security of supply and scalability. The conjugation protocol, buffer requirements, storage conditions, and the product sizes are identical.

Alkaline Phosphatase Conjugation Kit / Alkaline Phosphatase Labeling Kit ab102850 uses a simple and quick process for alkaline phosphatase 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 Alkaline Phosphatase using this kit:

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

The alkaline phosphatase 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 HRP.

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<sup>®</sup> Alkaline Phosphatase Labeling Kit. 702-0005 is the same as the 100  $\mu$ g size. 702-0010 is the same as the 3 x 100 ug size. 702-0030 is the same as the 3 x 10 ug size. 702-0015 is the same as the 1 mg size.

Amount and volume of antibody for conjugation to Alkaline Phosphatase

Kit size	Recommended maximum	Maximum antibody
	amount of antibody	volume <sup>1</sup>

拟丛

说明

3 x10 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

<sup>&</sup>lt;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>&</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

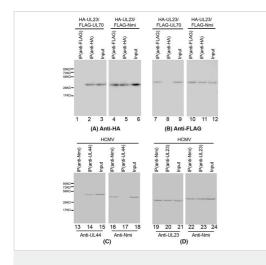
Lyophilized Lightning-Link<sup>®</sup> components are hygroscopic.

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.

#### 性能

组件	1 mg	100 μg	3 x 10 µg	3 x 100 µg
ab274118 - AP-Mix	1 x 1mg	1 x 100µg	3 x 10µg	3 x 100µg
ab274119 - Modifier reagent	1 x 200µl	1 x 200µl	1 x 200µl	1 x 200µl
Quencher reagent	1 x 200µl	1 x 200µl	1 x 200µl	1 x 200µl

### 图片



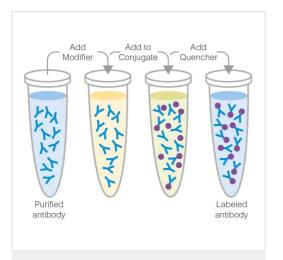
Western blot - Alkaline phosphatase Conjugation

# Kit;- Lightning-Link

Image from Feng, Linyuan, et al., PLoS pathog., 14(1): e1006867; doi: 10.1371/journal.ppat.1006867. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

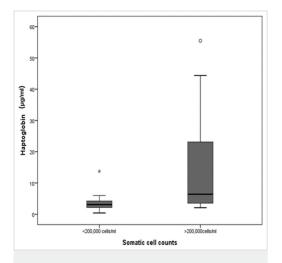
Feng, Linyuan, et al used Alkaline phosphatase Conjugation Kit - Lightning-Link® (ab102850) as part of examining the interaction between UL23 and Nmi identified by coimmunoprecipitation. They used the kit to conjugate Alkaline phosphatase to primary antibodies for use in western blot.

(A-B) Human U251 cells were co-transfected with a combination of two plasmids expressing FLAG- and HA-tagged proteins, and then harvested at 48 hours posttransfection. (C-D) Human U251 cells were infected with human cytomegalovirus (HCMV), a human herpesvirus, (MOI = 1) and cellular lysates were prepared at 48-72 hours postinfection. The input protein samples (80 µg) (Input) (lanes 3, 6, 9, 12, 15, 18, 21, and 24) and samples (15 µg) that were precipitated with anti-HA (IP (anti-HA)) (lanes 2, 5, 8, and 11), anti-FLAG (IP (anti-FLAG)) (lanes 1, 4, 7, and 10), anti-Nmi (lanes 13, 16, 19, and 22), anti-UL44 (lanes 14 and 17), or anti-UL23 antibodies (lanes 20 and 23), were separated on SDS-containing polyacrylamide gels, and assayed with Western blot analysis using anti-HA (anti-HA) (A), anti-FLAG (anti-FLAG) (B), anti-UL44 (anti-UL44) (C), anti-UL23 (anti-UL23) (D), and anti-Nmi (anti-Nmi) (C-D) antibodies that were directly conjugated to alkaline phosphatase using the Lightning-Link® kit ab102850, respectively.



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

### Alkaline Phospahatse Conjugation Kit



Alkaline phosphatase Conjugation Kit - Lightning-

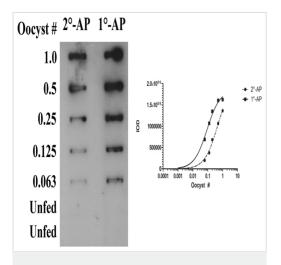
Link® labeling anti-bovine Hp antibody for ELISA

Image from Thomas FC et al., BMC Vet Res., 11, 207. Fig 1.; doi: 10.1186/s12917-015-0533-3. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

Thomas FC *et al* used ab102850 as part of examining the toxicity of p17 protein assemblies.

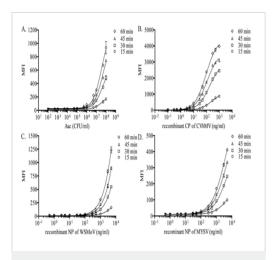
They used the kit to conjugate Alkaline phosphatase to anti-bovine Hp antibody for use in ELISA.

Boxplot showing Hp concentration ( $\mu$ g/ml) in two SCC categories of composite milk samples \* indicates an extreme value (values greater than 3 interquartile range (IQR) away from 25th or 75th percentile); IQR = 3rd quartile -1st quartile (represented by the height of the box). ° indicates an outlier value (values greater than 1.5 interquartile range (IQR) away from 25th or 75th percentile); IQR = 3rd quartile -1st quartile (represented by the height of the box).



Alkaline phosphatase Conjugation Kit - Lightning-Link® labeling anti P. falciparum circumsporozite protein antibody for ECL slot blot assay

Image from GrabiasB et al., PLoS One, 12(4): e0174229. Fig 3; doi: 10.1371/journal.pone.0174229. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/



Alkaline phosphatase Conjugation Kit - Lightning-Link® labeling antibodies for sandwich ELISA

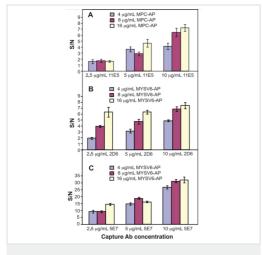
Image from Charlermroj R et al., PLoS One, 8(4): e62344. Fig 5; doi: 10.1371/journal.pone.0062344. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

Grabias B et al. used ab102850 as part of developing a rapid detection of *Plasmodium falciparum* infection in mosquitoes.

They used the kit to conjugate Alkaline phosphatase to anti-*Plasmodium falciparum* circumsporozite protein antibody for use in ECL slot blot assay.

Evaluation of whether conjugation of primary mAb 2A10 with alkaline phosphatase (1°-AP) enhanced sensitivity for the detection of Pfoocyst prepared from mosquito lysates when compared to the use of AP-conjugated secondary antibody (2°-AP). Detection limits were compared for each protocol by fitting the band intensities of serially diluted oocysts to a Michaelis-Menten regression curve and establishing a cutoff intensity threshold of mean + 2 SD from unfed mosquito specimens run on the same blot. Labeled primary antibody displayed overall higher band intensities across the range of oocyst dilutions examined and achieved lower limits of detection than the typical sandwich antibody format (0.009 oocyst versus 0.02 oocyst, respectively). The removal of an additional antibody incubation step also contributed to an overall shorter assay time in the newly developed slot blot protocol.

Charlermroj R et al. used ab102850 to run a sandwich ELISA and compare its sensitivity with a microsphere immunoassay based on Luminex using the same set of antibodies.



Thaitrong N et al. used ab102850 to run a microfluidic sandwich ELISA for Acidovorax citrulli (Ac), watermelon silver mottle virus (WSMoV), and melon yellow spot virus (MYSV) screening. Nine different conditions for each disease panel were tested on the microfluidic platform using combinations of three concentrations of capture Ab (11E5, 2D6, and 5E7) and three concentrations of detection Ab (MPC-AP, MYSV6-AP).

Alkaline phosphatase Conjugation Kit - Lightning-Link® labeling MPC and MYSV6 polyclonal antibodies for microfluidic sandwich ELISA

Image from Thaitrong N et al., PLoS One, 8(12): e83231. Fig.; doi: 10.1371/journal.pone.0083231. Reproduced under the Creative Commons license https://creativecommons.org/licenses/by/4.0/

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