

Biotinylation Kit / Biotin Conjugation Kit (Fast, Type A) - Lightning-Link® ab201795

[49 References](#) [10 Images](#)

Overview

Product name

Biotinylation Kit / Biotin Conjugation Kit (Fast, Type A) - Lightning-Link®

Product overview

Biotinylation Kit / Biotin Conjugation Kit (Type A) (ab201795) uses a simple and quick process for biotinylation / biotin labeling 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 Biotin using this kit:

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

The biotin 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 Biotin.

The Type A Biotinylation Kit / Biotin Conjugation Kit has been optimized to produce conjugates for assays in which a streptavidin-labeled detection reagent will be used.

Use the Type B Biotinylation Kit / Biotin Conjugation Kit [ab201796](#) for assays in which the biotinylated protein is captured by streptavidin immobilized on a surface (e.g. plates, nitrocellulose, magnetic beads etc).

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® Rapid Biotin Type A Labeling Kit. 370-0005 is the same as the 100 µg size. 370-0010 is the same as the 3 x 100 µg size. 370-0030 is the same as the 3 x 10 µg size. 370-0015 is the same as the 1 mg size.

Amount and volume of antibody for conjugation to Biotin

<i>Kit size</i>	<i>Recommended amount of antibody¹</i>	<i>Maximum amount of antibody</i>	<i>Maximum antibody volume²</i>

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

¹ Using the maximum amount of antibody may result in less labelling per antibody.

² Ideal antibody concentration is 1mg/ml. 0.5 - 1 mg/ml can be used if the maximum antibody volume is not exceeded. Antibodies > 2mg/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 ¹	0.1% BSA ²	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%

² BSA can also interfere with the use of the conjugated antibody in tissue staining.

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.

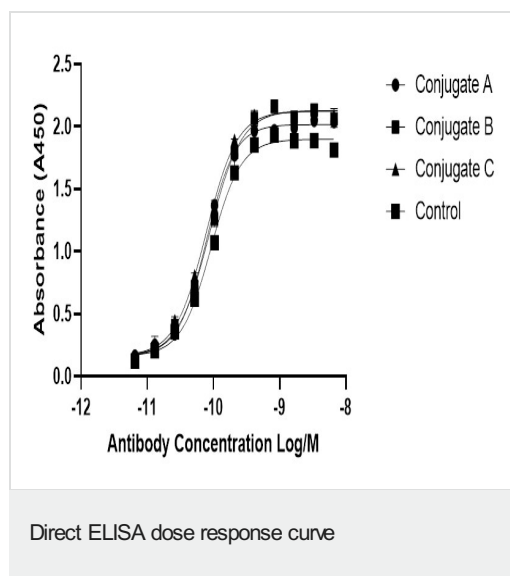
Kits are intentionally shipped at ambient temperature with silica gel to avoid exposure to moisture. Upon receipt, store the kit frozen and protect from moisture. Before opening the outer container, allow the lyophilized components to reach room temperature to minimize condensation.

Storage instructions

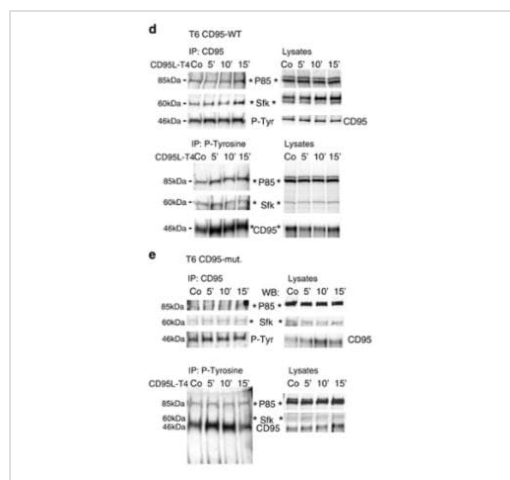
Store at -20°C. Please refer to protocols.

Components	1 mg	100 µg	3 x 100 µg	3 x 10 µg
ab274076 - Biotin (Type A) Conjugation Mix	1 x 1mg	1 x 100µg	3 x 100µg	3 x 10µg
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

Images



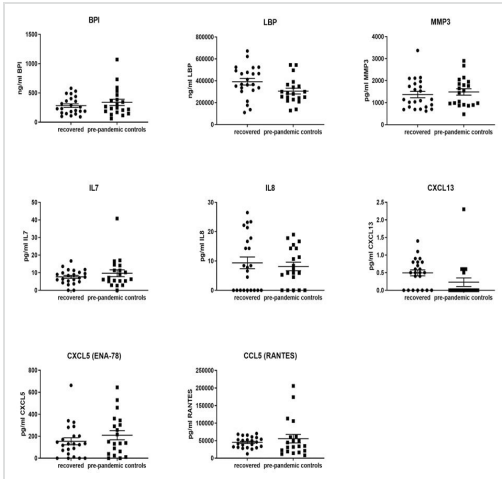
Direct ELISA dose response curve generated with varying concentration of biotin conjugated antibodies. [Ab242835](#), [ab242990](#), [ab242955](#) and [ab242950](#) were conjugated to biotin using Biotin Conjugation Kit (Fast, Type A) - Lightning-Link® ([ab201795](#)) to produce Conjugate A, B, C and Control respectively. Briefly, each well was coated with Recombinant Protein G. After blocking with 5% BSA blocking buffer for 2 hours, plates were coated with 1µg/ml of biotin conjugated antibodies. Detection was performed using a HRP-conjugated Streptavidin ([ab7403](#)), and visualised using a TMB substrate. Plates were read at 450nm using an Agilent BioTek Synergy HTX multimode reader.



Biotinylation Kit / Biotin Conjugation Kit (Fast, Type A) - Lightning-Link

Image from Drachsler, Moritz, et al., Cell death & disease 7.4 (2016): e2209-e2209. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

Drachsler, Moritz, et al used Biotinylation Kit / Biotin Conjugation Kit (Fast, Type A) - Lightning-Link® ([ab201795](#)) as part of examining expression of CD95. They used the kit to conjugate biotin to anti-human CD95 for use in Western blot. IP for CD95 and P-Tyrosine GSCs in naive, CD95-WT and CD95-mut GBM cells stimulated with CD95L-T4. Blots were probed with anti-P85 (regulatory PI3K subunit), anti-Sfk, anti-CD95 or anti-P-Tyrosine antibodies, respectively.

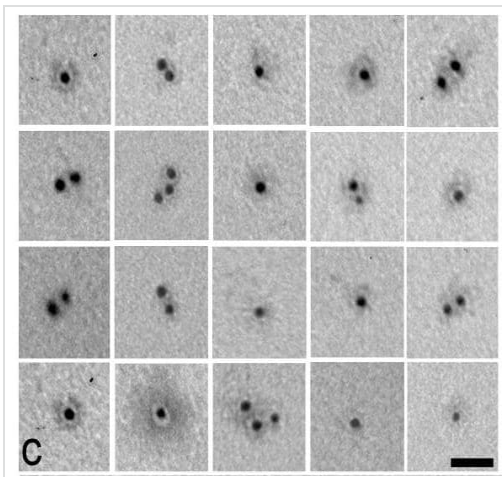


Multiplex Protein Detection - Biotinylation Kit / Biotin Conjugation Kit (Fast, Type A) Lightning-Link (ab201795)

Image from Hahnel, Viola, et al., PLoS one, 15(12): e0243967; doi: 10.1371/journal.pone.0243967. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

Hähnel, Viola, et al used Biotinylation Kit / Biotin Conjugation Kit (Fast, Type A) - Lightning-Link® (ab201795) as part of examining cytokine levels of plasma donors. They used the kit to conjugate Biotinylation Kit / Biotin to cytokine proteins for use in multiplex protein detection.

Cytokines, lipopolysaccharide binding protein (LBP) and bactericidal permeability increasing protein (BPI) of convalescent donors were compared to healthy control group. The following cytokines were below limit of detection: IL1β, IL1RA, IL2, IL3, IL6, IL10, IL12p40, IL15, IL21, IL22, IL23, IFNβ, IFNγ, GM-CSF, MIP1α and TNFα.

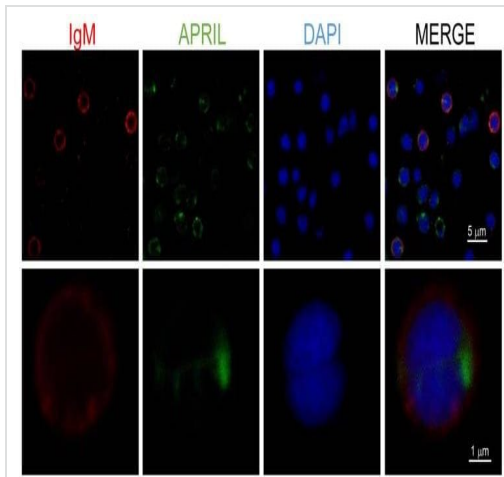


Electron Microscopy - Biotinylation Kit / Biotin Conjugation Kit (Fast, Type A) Lightning-Link

Image from Hansen, Uwe, et al., Plos one, 7(12): e52793.; doi: 10.1371/journal.pone.0052793. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

Hansen, Uwe, et al used Biotinylation Kit / Biotin Conjugation Kit (Fast, Type A) - Lightning-Link® (ab201795) as part of examining WARP-collagen IV interactions in in vitro in cartilage. They used the kit to conjugate biotin to recombinant WARP for use in Electron Microscopy.

Biotinylated recombinant WARP was visualized by 5 nm gold labeled with streptavidin. Scale bar is 25 nm

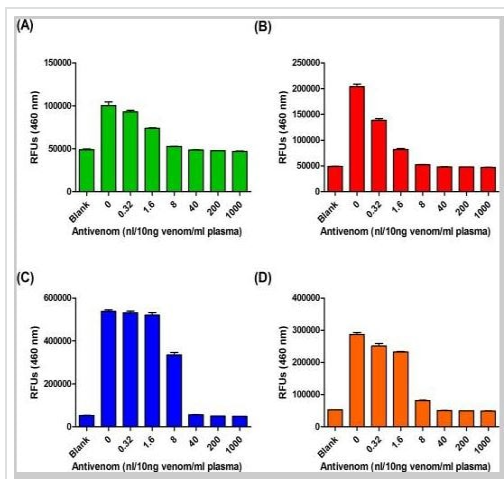


Fluorescence Microscopy - Biotinylation Kit / Biotin Conjugation Kit (Fast, Type A) - Lightning-Link

Image from Soleto, Irene, et al., *Frontiers in immunology*, 9:1880. doi: 10.3389/fimmu.2018.01880; Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

Soleto, Irene, et al used Biotinylation Kit / Biotin Conjugation Kit (Fast, Type A) - Lightning-Link® (ab201795) as part of examining the effect of APRIL on B cells using rainbow trout (*Oncorhynchus mykiss*) as a model species. They used the kit to conjugate biotin to a recombinant trout proliferation-inducing ligand (APRIL) for use in Fluorescence microscopy.

Total leukocytes from spleen were incubated with recombinant biotinylated APRIL (1 μg/ml) for 15 min, then plated onto poly-L-lysine-coated glass slides, fixed and labeled with anti-IgM (shown as red) and FITC-streptavidin (APRIL, shown as green), then counterstained with DAPI (blue), and analyzed by confocal fluorescence microscopy.

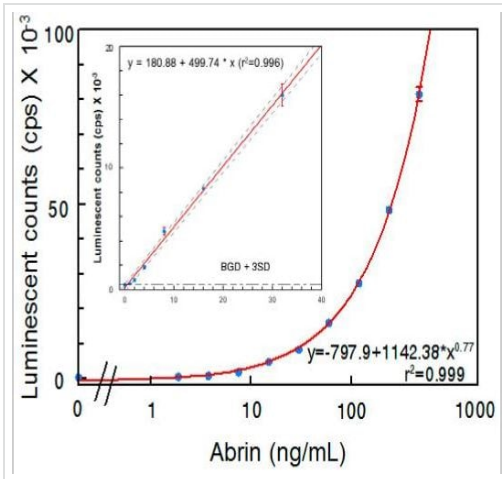


ELISA - Biotinylation Kit / Biotin Conjugation Kit (Fast, Type A)- Lightning-Link

Image from Liu, Chien-Chun, et al., *PLoS Negl Trop Dis*;12(12): e0007014. doi: 10.1371/journal.pntd.0007014. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

Liu, Chien-Chun, et al used Biotinylation Kit / Biotin Conjugation Kit (Fast, Type A) - Lightning-Link® (ab201795) as part of examining assays for snakebite detection based on clinical antivenom usage. They used the kit to conjugate biotin to neurotoxic species-specific antibodies (NSS-Abs) and hemorrhagic species-specific antibodies (HSS-Abs) for use in ELISA.

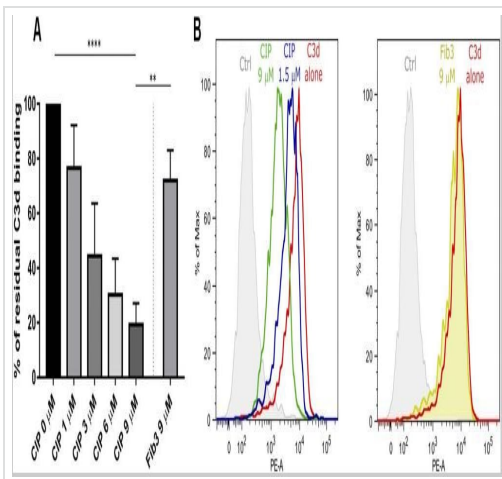
(A, B) Venom proteins of (A) *Trimeresurus stejnegeri* and (B) *Protobothrops mucrosquamatus* were diluted in human plasma (10 ng venom protein per ml of plasma) and then mixed with serially diluted Freeze-dried hemorrhagic antivenom (FHAV) (0.32 to 1000 nL) at room temperature for 30 min. The mixtures were then subjected to HSS-Ab–based sandwich ELISA assay. (C, D) Venom proteins of (C) *Bungarus multicinctus* and (D) *Naja atra* were diluted in human plasma (10 ng venom protein per ml of plasma) and then mixed with serially diluted FHAV (0.32 to 1000 nL) at room temperature for 30 min. The mixtures were then subjected to NSS-Ab–based sandwich ELISA assay.



ELISA - Biotinylation Kit / Biotin Conjugation Kit
(Fast, Type A) - Lightning-Link

Image from He, Xiaohua, et al., *Toxins* 9.12 (2017): 386. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

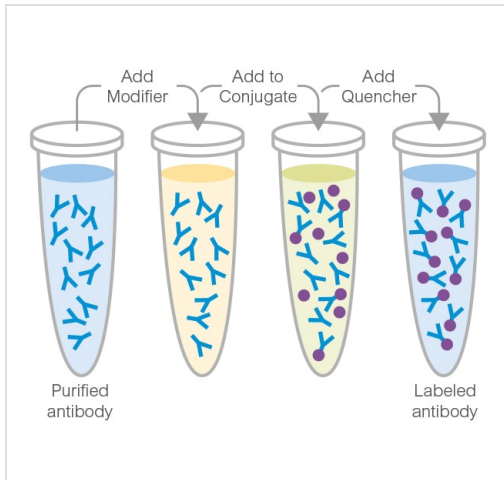
He, Xiaohua, et al used Biotinylation Kit / Biotin Conjugation Kit (Fast, Type A) - Lightning-Link® (ab201795) as part of examining the detection of abrin by monoclonal antibodies. They used the kit to conjugate biotin to anti-Abrin antibody for use in ELISA. Detection of abrin in PBS by ELISA. Data represent the average of three determinations ± SD. Limit of detection (LOD) for the mixture of abrin was 1 ng/mL. The one-sided 95% confidence intervals on the fitted line are shown as dashed curves.



Flow Cytometry - Biotinylation Kit / Biotin Conjugation Kit (Fast, Type A) - Lightning-Link

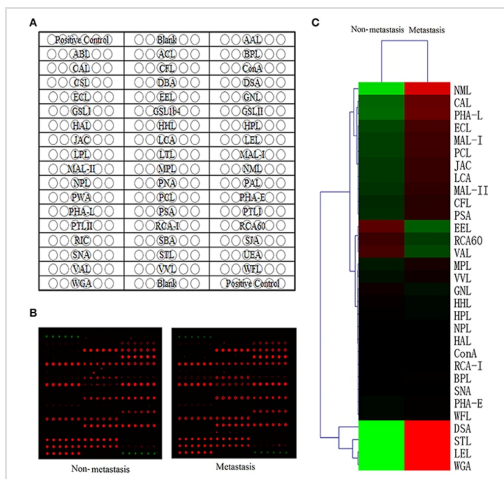
Image from Giussani, Stefania, et al., *The FASEB Journal* 33.3 (2019): 4448-4457. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

Giussani, Stefania, et al used Biotinylation Kit / Biotin Conjugation Kit (Fast, Type A) - Lightning-Link® (ab201795) as part of examining interaction of C3d ligand with complement interfering protein (CIP). They used the kit to conjugate biotin to C3d for use in flow cytometry. Flow cytometry analysis of C3d interaction with B cells in presence or absence of Complement Interfering Protein (CIP). A) Biotinylated C3d was preincubated with SA and then with 1–9 μM of CIP, 9 μM of Fib3, or buffer alone. Each mixture was added to Raji B cells and treated as described. Binding of the biotinylated C3d–SA complex to cells was revealed by flow cytometry using a C3d-specific mAb and a phycoerythrin-labeled secondary antibody. Mean fluorescence intensity values of the peaks were analyzed by the FlowJo software. The graph shows the percentage of the residual mean fluorescence intensity values in the presence of a competitor compared with buffer alone, as derived from 4 independent experiments. B) Flow cytometry analysis of C3d binding to enriched B cells from human PBMCs in presence or absence of 1.5 or 9 μM CIP or 9 μM of Fib3; experimental conditions were the same as in A.



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

Conjugation - Biotin (Type A) Conjugation Kit (ab201795)



Liu T et al. used ab201795 as part of examining glyco biomarker in hepatocellular carcinoma metastasis.

They used the kit to conjugate biotin to total proteins from lysate for use in a lectin microarray.

Lectin microarray analysis of glycoforms. (A) The lectin microarray contains 50 lectin spots with different binding specificities. (B) Scan image of the lectin microarray incubated with biotinylated proteins and Cy5 labeled streptavidin. Typical glycan profiles of HCC with metastasis were shown on the right and HCC with non-metastasis on the left. (C) Hierarchical clustering of positive lectin binding spots ($S/B \geq 2$). Each row represented a single lectin, S/B values were shown by the color scale: red represents a lectin with high S/B value while green represents a lectin with low S/B value.

Biotinylation Kit / Biotin Conjugation Kit (Type A) - Lightning-Link® labeling total proteins from lysate for a lectin microarray

Image from Liu T et al., Front Physiol., 8:472. Fig 1.; doi: 10.3389/fphys.2017.00472. Reproduced under the Creative Commons license <https://creativecommons.org/licenses/by/4.0/>

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