



LinKine™ AP Labeling Kit

Cat #: KTL0110

Size: 3×100 µg/1 mg

	AP Labeling Kit		
REF	Cat #: KTL0110	LOT	Lot #: Refer to product label
	Applicable samples: labeling biomolecules, especially proteins, peptides, and other ligands that contain a free amino group		
	Storage: Stored at 4°C for 6 months, protected from light		

Assay Principle

Alkaline phosphatase (ALP or AKP) is an enzyme that can dephosphorylate the corresponding substrate by hydrolyzing the phosphate monoester to remove the phosphate group on the substrate molecule and generate phosphate ions and free hydroxyl groups. Such substrates include nucleic acid, protein, alkaloid, etc. And the process of removing phosphate group is called dephosphorylation. Alkaline phosphatase is a kind of phosphatase, which can be combined with the molecule to be labeled. When incubated with an appropriate substrate, it will generate a labeled molecule conjugate with a color, fluorescent or luminescent derivative, which can be detected and quantitatively analyzed. AP is usually used in conjugates to determine the existence of molecular targets, and is often used in ELISA, Western blot and immunohistochemistry. Since higher concentrations of inorganic phosphorus can competitively inhibit the activity of alkaline phosphatase, systems such as phosphate-rich PBS should not be used in the immune experiment, otherwise it will cause a high background, and TBS and other systems without phosphate can be used.

LinKine™ AP Labeling Kit uses glutaraldehyde as a cross-linking agent to bind the amino groups of the enzyme and antibody protein to the two aldehyde groups of glutaraldehyde by one-step method. AP can be directly labeled on proteins, peptides and other ligands containing free amino groups. The coupling product can be stored stably for 1 month at 4°C.

Application examples: Directly labeled primary antibodies are advantageous as they eliminate the need for secondary reagents in immunoassay procedures, thus removing a tedious extra cycle of incubation and wash steps in applications.

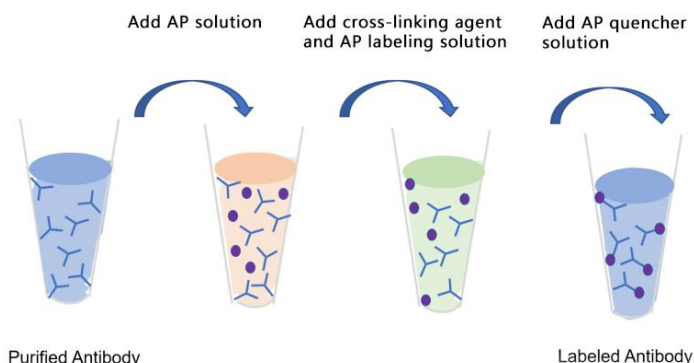


Figure 1: Schematic diagram of LinKine™ AP Labeling Kit

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	3×100 µg	1 mg	
AP Solution	30 µL	100 µL	4°C, protected from light
AP Labeling Solution	10 mL	10 mL	4°C
Cross-linking Agent	500 µL	500 µL	4°C, protected from light
AP Quencher Powder	3	1	4°C, protected from light
Booklet	1	1	RT

Materials Required but Not Supplied

- Prepared samples to be labelled, deionized water
- Precision pipettes, disposable pipette tips, ultrafiltration tubes (if needed)

Sample Preparation

List of components (or storage buffer) requirements of the sample to be labeled:

pH	6.5-8.0
Amine Free Buffer	MES, TBS, HEPES
Chelating Agents (e. g. EDTA)	✓
Glycerol	< 50%
BSA	< 0.1%
Glycine	X
Gelatin	< 0.1%
Proclin	X
Phosphate Buffer	X
Components Containing Amino	X

Assay Procedure

1. Setting up your conjugation reactions

The best molar ratio of the sample to be labeled to AP is 1:1 to 1:2. We recommend to perform coupling experiments at a ratio of approximately 1:1. You can determine the optimal molar ratio through preliminary experiments. Taking labeled antibody as an example, the recommended dosage from 1:1 to 1:2 is as follows.

Size	Amount of AP	Amount of antibody (Ab: AP)		Optimal Labeling system
		1:1	1:2	
3×100 µg/Kit	300 µg	300 µg	150 µg	150 µL
1 mg/Kit	1 mg	1 mg	0.5 mg	0.5 mL

Taking account of the AP molecular weights (160,000 versus 160,000), this means for that for 1 mg AP you need to add 0.5-1 mg of antibody.

Replace the buffer for the sample to be labeled with AP labeling solution. Ultrafiltration is recommended to fill up the AP labeling

solution to 500 μ L, centrifuge at 4°C, 12,000 g for 10 min, replenish AP labeling solution to 500 μ L, and centrifuge 12,000 g at 4°C until the sample volume does not exceed 50 μ L. After centrifugation, the inner tube of the ultrafiltration tube is taken out, inverted inner tube of ultrafiltration tube in a clean outer tube, centrifuge at 4,000 g, 4°C, 2 min to collect the sample to be labeled. Take the 3 \times 100 μ g/Kit for example, the recommended ratio of antibody to be labeled and AP enzyme is 1:1. For other sizes, the amount of the components will be adjusted in equal proportion referring to the procedures. If you want to adjust the molar ratios, keeping the amount of AP constant, and you could change the amounts of molecules and buffer dosages according to the above table.

(1) Add 30 μ L of AP solution to the sample to be labeled. Mix gently using a pipette.

(2) Add 15 μ L cross-linking agent to the solution in step (1), add AP labeling solution to 150 μ L. Mix gently and react for 2 h in the dark at room temperature on a vertical mixer.

(3) Add 1 mL AP labeling solution to AP quencher powder to make AP quencher solution. The AP quencher solution could be placed at room temperature, but should be used within 24 h.

Note: Please prepare the AP quencher solution with lid open to prevent tube burst, due to gas accumulation in the tube and expand instantly; The dosage of AP labeling solution for preparing AP quencher solution with different sizes of kits remains unchanged (1 mL).

(4) Add 60 μ L AP quencher solution to the solution of Step (2), mix gently and standing for 1 h at 4°C avoiding the light.

(5) Ultrafiltration is used to remove small molecular impurities. Fill up the AP labeling solution to 500 μ L, centrifuge at 4°C, 12,000 g for 10 min. Replenish AP labeling solution to 500 μ L again and centrifuge 12,000 g at 4°C for 10 min (The time can be appropriately extended to make the volume smaller and the conjugate concentration high). After centrifugation, the inner tube of the ultrafiltration tube is taken out, inverted inner tube of ultrafiltration tube in a clean outer tube, centrifuge at 4,000 g, 4°C, 2 min to collect the sample to be labeled.

(6) The conjugates after removing small molecular impurities can be directly used in the next experiments.

2. Storage of conjugates

The conjugate can be stored stably for one month at 4°C in the dark. For long-term storage, please separate and store at -20°C in the dark. Avoid repeated freezing and thawing. We recommend to add the same volume of glycerin to the conjugate. If you need to dilute the conjugate, avoid using buffers such as phosphate-rich PBS, and use buffers that do not contain phosphate such as TBS.

FAQ

Q1: The reaction volume exceeds the optimal coupling system because of low concentration of antibodies?

A1: If the concentration is still less than 2 mg/mL after the concentrate, adjust the reaction volume properly, but the final concentration should be more than 1 mg/mL. The ratio of AP solution to Quencher solution should remain unchanged.

Q2: The molar ratios of molecules with AP can only between 1:1 and 1:2?

A2: For different molecules, the suitable molar ratios are disparate. For antibodies, we suggest it should not exceed 1:2. For other molecules, you could try unlike ratios according to your own requirements

Precautions

1. Different batch number, different manufacturers of components do not mix; Otherwise, the result may be abnormal.
2. When mixing or reconstitution, avoid bubbles. If there are unused reagents, please seal and store at 4°C in time.
3. Frequently change the pipette tip to avoid cross-contamination between components.
4. Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.

Recommended Products

Catalog No.	Product Name
-------------	--------------

CTL0100	LinKine™ HRP Labeling Kit
CTL0210	LinKine™ FITC Labeling Kit
CTL0520	LinKine™ AbFluor™ 488 Labeling Kit
CTL0540	LinKine™ AbFluor™ 594 Labeling Kit
CTL0120	LinKine™ Biotin Labeling Kit
CTL0530	LinKine™ AbFluor™ 555 Labeling Kit
CTL0560	LinKine™ AbFluor™ 647 Labeling Kit
CTL0580	LinKine™ AbFluor™ 680 Labeling Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.