# FlyLink™ AP Labeling Kit

Cat #: D-AKE110

Size: 100 μg x 3

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.

FlyLink™ 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.

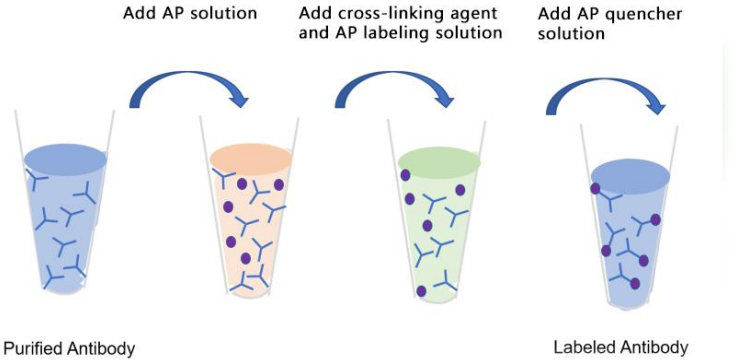


Fig.1: Schematic diagram of FlyLink™ AP Labeling Kit

**Applicable samples:** labeling biomolecules, especially proteins, peptides, and other ligands that contain a free amino group.

## Materials Supplied and Storage Conditions

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

## Materials Required but Not Supplied

·Prepared samples to be labelled, Deionized water

·Precision pipettes, Disposable pipette tips, Ultrafiltration tubes or dialysis bags (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 | ✗ |
| Gelatin | < 0.1% |
| Proclin | ✗ |
| Phosphate Buffer | ✗ |
| Components Containing Amino | ✗ |

## 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 μL, centrifuge at 4°C, 12,000 g for 10 min,replenish AP labeling solution to 500 μL, and centrifuge 12,000 g at 4°C until the sample volume does not exceed 50 μL. 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 3×100 μg/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 μL of AP solution to the sample to be labeled. Mix gently using a pipette.

(2) Add 15 μL cross-linking agent to the solution in step (1), add AP labeling solution to 150 μL. 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 μL 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 μL, centrifuge at 4°C, 12,000 g for 10 min. Replenish AP labeling solution to 500 μL 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 HRP can only between 1:1 and 1:4?**

**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 rations according to your own requirements.

## Disclaimer

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