ab102850
Alkaline Phosphatase Conjugation Kit Protocol

Instructions for Use
Antibody and protein modification
This product is for research use only and is not intended for diagnostic use.
INTRODUCTION

1. BACKGROUND

Abcam's Alkaline Phosphatase Conjugation Kit provides a simple and quick process to conjugate your primary antibodies with alkaline phosphatase. The conjugated antibody can be used straight away in WB, ELISA, IHC etc. The antibody to be labelled should be purified, in an appropriate buffer for conjugation and at a suitable concentration, as described in section 6. If not, consider using our antibody purification and concentration kits. http://www.abcam.com/kits/antibody-purification-and-concentration-kits

The Kit is available in 4 sizes

- 30µg (3 x 10 µg) sufficient for labeling up to 30µg of antibody
- 100µg (1 x 100 µg) sufficient for labeling up to 100µg of antibody
- 300µg (3 x 100µg) sufficient for labeling up to 300µg of antibody
- 1mg (1 x 1mg) sufficient for labeling up to 1mg of antibody
2. ASSAY SUMMARY

Add Modifier
Add to Conjugate
Add Quencher

Purified antibody

Labeled antibody
3. PRECAUTIONS
Please read these instructions carefully prior to beginning the assay.
All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Storage of Kit
Observe the storage conditions for individual prepared components in section 6 Materials Supplied.
Aliquot components in working volumes before storing at the recommended temperature.

Storage of new conjugate
For any new conjugate, initial storage at 4°C is recommended for 12-18 months*. A preservative may be desirable for long-term storage up to 2 years. Other storage conditions (e.g. frozen at -70°C or stored at -20°C with 50% glycerol) may also be satisfactory. The best conditions for any particular conjugate must be determined by experimentation.
*As long as the antibody can be stored at 4°C – check the manufacturer’s recommendation.

5. LIMITATIONS
- Conjugation kit intended for research use only.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
### 6. MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 µg (3 x 10 µg)</td>
<td>-20°C</td>
</tr>
<tr>
<td>AP Mix</td>
<td>3x10 µg</td>
<td>-20°C</td>
</tr>
<tr>
<td></td>
<td>100 µg (1 x 100 µg)</td>
<td>-20°C</td>
</tr>
<tr>
<td>Quencher</td>
<td>1 Vial</td>
<td>-20°C</td>
</tr>
<tr>
<td></td>
<td>300 µg (3 x 100 µg)</td>
<td>-20°C</td>
</tr>
<tr>
<td>Modifier</td>
<td>1 Vial</td>
<td>-20°C</td>
</tr>
<tr>
<td></td>
<td>1 mg (1 x 1 mg)</td>
<td>-20°C</td>
</tr>
<tr>
<td></td>
<td>1 Vial</td>
<td>-20°C</td>
</tr>
</tbody>
</table>
7. MATERIALS REQUIRED NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- Unconjugated antibody or protein
- Microfuge tubes (0.5 or 1.5 mL)
- Microfuge
- Adjustable pipette

8. TECHNICAL HINTS

- Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening to avoid reagent loss in tube caps.

10. SAMPLE PREPARATION

Pre-conjugation Considerations:

10.1. The purified antibody to be labeled should ideally be in 10 – 50 mM amine-free buffer (e.g. MES, MOPS, HEPES, PBS), pH range 6.5 to 8.5.

10.2. Common non-buffering salts (e.g. sodium chloride), chelating agents (e.g. EDTA), and sugars have no effect on conjugation efficiency. Azide (0.02 to 0.1%) and BSA (0.1 to 0.5%) have little or no effect. Glycerol up to 50% has no effect.

10.3. Avoid buffer components that are nucleophilic, as these may react with Fast Conjugation Kit chemicals. Primary amines (e.g. amino acids, ethanolamine or Tris) and thiols (e.g. mercaptoethanol or DTT) fall within this class. Thimerosal (thiomersal) should also be avoided.

10.4. Recommended amount and volume of antibody for optimal results:

<table>
<thead>
<tr>
<th>Vial Size</th>
<th>Amount of Antibody (µg)</th>
<th>Volume of Antibody (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 µg</td>
<td>10 - 20</td>
<td>4 - 10</td>
</tr>
<tr>
<td>100 µg</td>
<td>100 - 200</td>
<td>40 - 100</td>
</tr>
<tr>
<td>1 mg</td>
<td>1000 - 2000</td>
<td>400 - 1000</td>
</tr>
</tbody>
</table>

Antibody concentrations of 1-4 mg/mL generally give optimal results.
11. CONJUGATION PROCEDURE

11.1. Add 1 µL of Modifier Reagent to each 10 µL of antibody to be labelled, mix gently.

11.2. Remove cap from vial of AP Mix and pipette the antibody sample (with added Modifier reagent) directly onto the lyophilized material. Resuspend gently by withdrawing and re-dispensing the liquid once or twice using a pipette.

11.3. Replace cap on the vial and leave standing for 3 hours in the dark at room temperature (20-25°C). Conjugations can also be set up and left overnight; longer incubation times have no negative effect on the conjugate.

11.4. After incubating for 3 hours (or more), add 1 µL of Quencher reagent for every 10 µL of antibody used and mix gently. The conjugate can be used after 30 minutes. The conjugates do not require purification.

11.5. Storage at 4°C is recommended for any conjugate. A preservative may be desirable for long-term storage.
12. **FAQ**

1. **What is the optimal starting concentration for the antibody?**
   The antibody conjugation kit allows antibody labeling to be performed on a microgram to milligram scale. The amount of antibody should correspond to molar ratios between 1:4 and 1:1 of antibody to conjugate. Based on their molecular weights (e.g. 160kDa for the antibody versus 150kDa for the conjugate), 100-400μg of conjugate can be added to 100μg of antibody. Antibody concentrations of 0.5-5mg/ml give optimal results. We recommend using 10μl, 100μl and 1ml of antibody solution with the 10μg, 100μg and 1mg kit formats, respectively. The antibody concentration for each conjugation kit has been optimised. Please refer to the relevant datasheet or protocol for the recommended antibody concentration.

2. **Do I need to purify the antibody before using the conjugation kit?**
   Yes. The antibody labeling chemistry involves free amine groups. Most proteins/peptides have lysine and/or alpha-amino groups, therefore, any protein/peptide present in the solution will also be labeled. We recommend purifying your antibodies before performing the conjugation. Ascites fluid, serum or hybridoma culture media should be avoided. View compatible and incompatible buffers in question 5.

3. **Are conjugation kits suitable for proteins and secondary antibodies?**
   Yes. The labeling chemistry involves free amines present in lysines and at the N-terminus of a protein. All antibodies have multiple free amine groups and most proteins have lysine and/or alpha-amino groups. As long as lysines are present, secondary antibodies and proteins will be labeled with the conjugation kits. However, they have not yet been specifically tested with secondary antibodies and proteins.
4. **What buffers can be used?**

We recommend using Hepes, MES, MOPS and phosphate-based buffers or any other amine-free buffer. Conjugation reactions can also be prepared in the presence of up to 20mM Tris buffer with almost no reduction in coupling efficiency. Once the reaction is complete, the conjugated antibody can be diluted in any buffer compatible with both label and antibody.

5. **Which buffer additives can be used and what should be avoided?**

Additives such as salts (e.g. NaCl), sugars (e.g. sucrose) and chelators (e.g. EDTA) have no effect on the labeling reaction. We recommend avoiding nucleophiles such as amino acids (e.g. glycine), blockers (e.g. ethanolamine) and thiols (DTT, mercaptoethanol) that might deactivate the chemical which covalently links the conjugate to the antibody.

**Compatible additives:**

- up to 20mM Tris
- up to 0.5% BSA
- up to 0.1% gelatin
- up to 0.1% sodium azide
- PBS pH7.4
- up to 50% glycerol
- 0.15M sodium chloride
- 0.02M potassium phosphate
- 0.001% Tween
- Proclin 300
- 5% Trehalose
- 0.15M sodium chloride
- 50mM HEPES
- 0.02M potassium phosphate
- 0.001% Tween
- Proclin 300
- 5% Trehalose
Incompatible additives:

- 60mM citrate + 150mM Tris pH7.8
- Urea
- 50mM Imidazole
- Glycine
- Ethanolamine
- DTT
- Mercaptoethanol

6. How do I remove additives from the antibody storage buffer?

Our Antibody Concentration and Purification kits remove additives with ease and provide a ready-to-use antibody solution compatible with the conjugation kit.

The Antibody Concentration kit allows an easy concentration and reduction of azide, glycine and Tris.

The Antibody Purification Kit quickly removes BSA, glycine, Tris, azide etc. and can also be used to purify antibodies from ascites fluid or immune serum.

For further FAQs visit: www.abcam.com/conjugationFAQS
13. PUBLICATIONS

Label: Cy3®  Application: FRET  
Effects of pH on molecular mechanisms of chitosane-integrin interactions and resulting tight-junction disruptions  

Label: R-PE  Application: Immunocytochemistry and In-vivo imaging*  
Intraoperative Imaging of Metastatic Lymph Nodes Using a Fluorophore-conjugated Antibody in a HER2/neu-expressing Orthotopic Breast Cancer Mouse Model  

*N.B. No product warranty for this application

Label: APC/Cy7®  Application: Flow cytometry  
APR-246/PRIMA-1^{MET} rescues epidermal differentiation in skin keratinocytes derived from EEC syndrome patients with p63 mutations  

Label: FITC  Application: Flow cytometry and cellular assay  
Constitutive dimerization of glycoprotein VI (GPVI) in resting platelets is essential for binding to collagen and activation in flowing blood.  
14. NOTES