Instructions for Use
Antibody and protein modification

This product is for research use only and is not intended for diagnostic use.
# Table of Contents

## INTRODUCTION
1. BACKGROUND 1  
2. SUMMARY 2  

## GENERAL INFORMATION
3. PRECAUTIONS 3  
4. STORAGE AND STABILITY 3  
5. LIMITATIONS 3  
6. MATERIALS SUPPLIED 4  
7. MATERIALS REQUIRED NOT SUPPLIED 5  
8. TECHNICAL HINTS 5  

## PREPARATION
9. REAGENT PREPARATION 6  
10. SAMPLE PREPARATION 6  

## PROCEDURE
11. PROCEDURE 7  

## RESOURCES
12. NOTES 8  

INTRODUCTION

1. BACKGROUND

Abcam’s R-Phycoerythrin Conjugation Kit provides a simple and quick process to conjugate your primary antibodies with R-PE. R-PE is an orange fluorescent dye has excitation wavelengths of 496 nm, 535 nm and 566 nm and an emission wavelength of 575 nm.

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 the 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

- 30 μg (3 x 10 μg) sufficient for labeling up to 30 μg of antibody
- 60 μg (1 x 60 μg) sufficient for labeling up to 60 μg of antibody
- 180 μg (3 x 60 μg) sufficient for labeling up to 180 μg of Antibody
- 600 μg (1 x 600 μg) sufficient for labeling up to 600 μg of antibody
2. SUMMARY

Purified antibody

Add Modifier

Add to Conjugate

Add Quencher

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
Store kit as given in the table upon receipt.
Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 6.
Aliquot components in working volumes before storing at the recommended temperature.

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.
## GENERAL INFORMATION

### 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></td>
</tr>
<tr>
<td>R-PE Mix</td>
<td>3x10 µg</td>
<td>-20°C</td>
</tr>
<tr>
<td></td>
<td>60 µg (1 x 60 µg)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1x100 µg</td>
<td>-20°C</td>
</tr>
<tr>
<td></td>
<td>180 µg (3 x 60 µg)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3x100 µg</td>
<td>-20°C</td>
</tr>
<tr>
<td></td>
<td>600 µg (1 x 600 µg)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1x1 mg</td>
<td>-20°C</td>
</tr>
<tr>
<td>Modifier Reagent</td>
<td>1 Vial</td>
<td>-20°C</td>
</tr>
<tr>
<td>Quencher Reagent</td>
<td>1 Vial</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

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Discover more at www.abcam.com
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>Kit size</th>
<th>Vial Size</th>
<th>Amount of Antibody (µg)</th>
<th>Volume of Antibody (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 µg</td>
<td>3x10 µg</td>
<td>3x10</td>
<td>3x10</td>
</tr>
<tr>
<td>60 µg</td>
<td>100 µg</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>180 µg</td>
<td>3x100 µg</td>
<td>3x60</td>
<td>3x60</td>
</tr>
<tr>
<td>600 µg</td>
<td>1 mg</td>
<td>600</td>
<td>600</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 R-PE Conjugation 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.