

ab201807 – APC Conjugation Kit

Instructions for Use

For the Covalent Conjugation of Antibodies or Proteins to Allophycocyanin (APC).

This product is for research use only and is not intended for diagnostic use.

Table of Contents

INTRODUCTION

- 1. BACKGROUND** 2
- 2. ASSAY SUMMARY** 3

GENERAL INFORMATION

- 3. PRECAUTIONS** 4
- 4. STORAGE AND STABILITY** 4
- 5. MATERIALS SUPPLIED** 4
- 6. MATERIALS REQUIRED, NOT SUPPLIED** 5
- 7. LIMITATIONS** 5
- 8. TECHNICAL HINTS** 5

ASSAY PREPARATION

- 9. REAGENT PREPARATION** 6
- 10. SAMPLE PREPARATION** 6

ASSAY PROCEDURE

- 11. ASSAY PROCEDURE** 7

RESOURCES

- 12. NOTES** 8

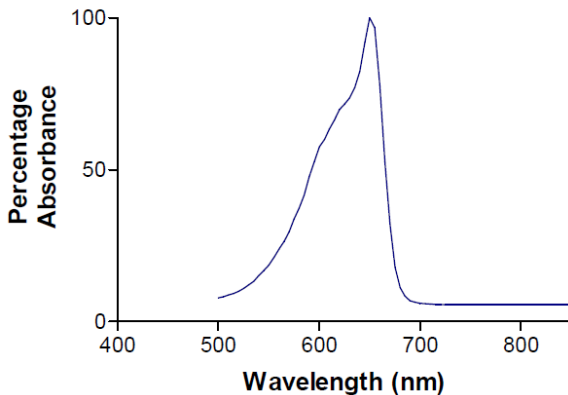
1. BACKGROUND

The Abcam APC Conjugation Kit (ab201807) provides an easy-to-use, one step procedure that allows researchers to covalently label proteins, peptides and other biomolecules containing primary amines with allophycocyanin (APC) with only 30 seconds hands-on time; furthermore conjugates are ready to use in 3 ½ hours.

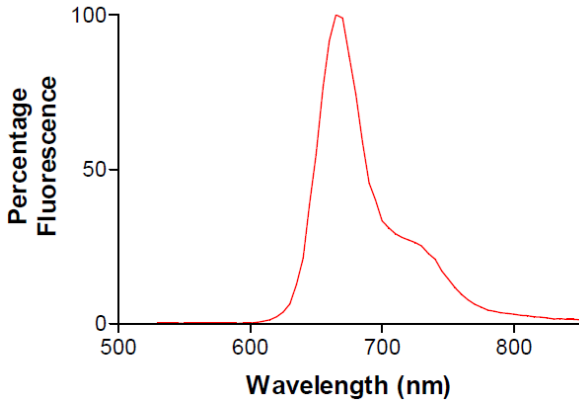
The antibody to be labelled should be purified, in an appropriate buffer for conjugation and at a suitable concentration, as described in section 10. If not, consider using our antibody purification and concentration kits.

APC is an accessory protein to chlorophyll from the phycobiliprotein family. APC is a 105 kDa protein that has six phycocyanobilin chromophores per molecule. APC has an absorption maximum at 650 nm and an emission maximum at 670nm.

Excitation scan of APC



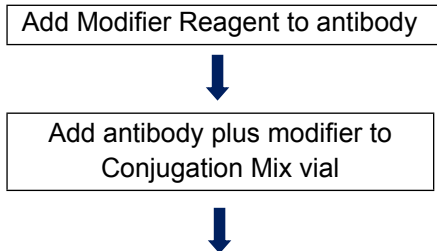
Emission scan of APC



The kit comes in 4 sizes for labelling recommended amounts of antibody:

- 100 μg containing 1 reaction (for 100 - 150 μg)
- 300 μg containing 3 reactions (each for 100 - 150 μg)
- 30 μg containing 3 reactions (each for 10 - 15 μg)
- 1 mg containing 1 reaction (for 1 - 1.5 mg)

2. ASSAY SUMMARY



Antibody labelled

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 at -20°C upon receipt.

Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	100 µg (1 x 100 µg)	300 µg (3 x 100 µg)	30 µg (3 x 10 µg)	1 mg (1 x 1 mg)	Storage Condition (Before Preparation)
APC Conjugation Mix	1 Vial	3 Vials	3 Vials	1 Vial	-20°C
APC Modifier Reagent	1 Vial	1 Vial	1 Vial	1 Vial	-20°C
APC Quencher Reagent	1 Vial	1 Vial	1 Vial	1 Vial	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Microfuge Tubes (0.5 or 1.5 mL)
- Microfuge
- Adjustable pipette or multiple-channel pipette

7. LIMITATIONS

- This kit intended for research use only. Not for use in diagnostic procedures
- 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

8. TECHNICAL HINTS

- 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.
- **Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Prepare fresh reagents immediately prior to use.

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 (< 0.1%) and BSA (<0.1%) have little or no effect. Glycerol <50%, Tris <20 mM and gelatin <0.1% have no effect.
- 10.3 Avoid buffer components that are nucleophilic, as these may react with Conjugation Kit chemicals. Compounds containing primary amines (e.g. amino acids, ethanolamine and Tris) and thiols (e.g. mercaptoethanol or DTT) fall within this class. Thimerosal (thiomersal, Merthiolate) should also be avoided.
- 10.4 Recommended amount and volume of antibody for optimal results:

Vial Size	Amount of Antibody (µg)	Volume of Antibody (µL)
10 µg	10 - 15	4 - 10
100 µg	100 - 150	40 - 100
1 mg	1000 - 1500	400 - 1000

When using the upper amount of antibody for each vial size (e.g. 15 µg of antibody for the 10 µg vial), each antibody molecule will be labeled with a single APC molecule (on average).

Antibody concentrations of 1 - 2.5 mg/mL generally give optimal results.

If intending to use the conjugated antibody produced using this kit for immunohistochemistry, it is recommended that is no gelatin or BSA present.

11. ASSAY PROCEDURE

- 11.1 Add 1 μL of APC Modifier reagent to each 10 μL of antibody to be labelled and mix gently.
- 11.2 Remove cap from vial of APC Conjugation Mix and pipette the antibody sample (with added APC 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 conjugation.
- 11.4 After incubating for 3 hours (or more), add 1 μL of APC 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.

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

12. NOTES

RESOURCES

RESOURCES

UK, EU and ROW

Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria

Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France

Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany

Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain

Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland

Email: technical@abcam.com

Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America

Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada

Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific

Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan

Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp

Copyright © 2015 Abcam, All Rights Reserved. The Abcam logo is a registered trademark.

All information / detail is correct at time of going to print.