

## ab288108 – Antibody Cy3 Labeling Kit

An easy way to label antibodies with Cy3 in a user-friendly spin column format.  
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab288108>

### Storage and Stability

- Store the kit at 4°C, protected from light.
- Read the entire protocol before performing the experiment.
- Briefly spin small vials prior to opening.
- Bring the kit components to room temperature before use.

### Materials Supplied

Item	Quantity	Storage Condition
EZLabel Cy3	5 Vials	4°C
EZLabel Spin Column	10 Columns	4°C
EZLabel Elution Buffer	10 mL	4°C

### Materials Required, Not Supplied

- Microcentrifuge
- DMSO/DMF
- Fresh 0.1 M Sodium Bicarbonate buffer (pH 8.5 - 9.0).

### Protocol

#### 1. Antibody Solution Preparation:

- The volume of antibody solution should not exceed 100 µl. For best results, use 100 µl of ~5-10 mg/ml antibody.

Δ **Note:** Buffers that contain primary amines (e.g. Tris or glycine) interfere with the intended Cy3 conjugation. Dialyze the antibody against 0.1 M sodium bicarbonate buffer (pH 8.5-9.0) just before labelling experiment is performed to remove the primary amines.

#### 2. Labelling reaction

- Each vial of EZLabel Cy3 is sufficient for labelling of 1 mg of antibody.
- Reconstitute one vial of EZLabel Cy3 with 5-10 µl of DMSO or DMF just before use. Dissolve completely by pipetting up and down.
- Transfer 100 µl of the prepared antibody to a 1.5 ml microcentrifuge tube.
- Add the reconstituted EZLabel Cy3 solution and mix well by pipetting up and down.
- Incubate at room temperature on rotary shaker or mixer for 1 hr. Total volume at this stage should not exceed 110 µl.

Δ **Note:** If the amount of antibody is less than 1 mg, the amount of EZLabel Cy3 also needs to be lowered accordingly to avoid over labeling of antibody

#### 3. Purification of Labeled Antibody

- During the labelling reaction, snap off the bottom closure of an EZLabel Spin Column and place in a fresh microcentrifuge tube. Centrifuge at ~1500 x g for 1 min. to remove the residual storage buffer. Discard the flow through and wash the resin with 110 µl of EZLabel Elution Buffer. Close the cap and centrifuge at 1500 x g for 1 min. Discard the flow through. Repeat this washing step for at least a total of three times.
- Load the labelling reaction mix (max. 110 µl) to the first spin column drop by drop. Centrifuge the column for 2 min. at 1500 x g to collect the eluant.
- Transfer the eluant onto the second unused spin column drop by drop. Centrifuge the column for 2 min. at 1500 x g to collect the labeled antibody.

- Optional:** Dialyze the labeled antibody in the dark against a desired storage buffer containing 20-30% glycerol and if necessary, add carrier protein (e.g. BSA) after the dialysis. Store the dialyzed antibody in a tube wrapped with aluminium foil at 4°C (for short term) or -20°C (for long term).

### Calculation

In some cases, it is advantageous to determine the number of molecules of Cy3 per molecule of antibody (degree of labelling). For that, measure the absorbance of the labelled antibody at 280 nm (A<sub>280</sub>) and 550 nm (A<sub>550</sub>). If necessary, dilute the labelled antibody in EZLabel Elution Buffer. Calculate the concentration of labelled antibody and degree of labelling using following equations:

$$\text{Conc. of labeled Antibody (M)} = \frac{A_{280} - (A_{550} \times 0.08)}{203000} \times \text{Path Length Correction} \times \text{Dilution Factor}$$

$$\# \text{ of moles Cy3 per mole Antibody} = \frac{A_{550} \times \text{Dilution Factor} \times \text{Path Length Correction}}{150000 \times \text{Antibody Concentration (M)}}$$

### Technical Support

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