AcalephFluor®350 Conjugation Kit

User Manual

Catalog # CRG1050

AcalephFluor®350 Conjugation Kit uses a simple and quick process for AcalephFluor®350 labeling/conjugation of antibodies. It can also be used to conjugate other proteins or peptides.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

AcalephFluor®350 Conjugation Kit provides a simple and rapid procedure to covalent labeling of antibodies with AcalephFluor®350 by targeting primary amine groups. It takes only 30 seconds hands-on time and conjugates are ready to use in less than 30 minutes without loss of antibodies.

This protocol is optimized for conjugation of IgG antibodies, for other proteins and biomolecules containing amine groups, please consult our guide.

The antibody to be labeled should be purified, in an appropriate buffer for conjugation and at a suitable concentration conjugation.

II. KIT COMPONENTS

Component	1 mg Size	5 mg Size	Storage
Reaction Buffer	1 ml x 1	5 ml x 1	4 °C
AcalephFluor®350	Powder x 1	Powder x 1	4 °C
DMSO	50 µl x 1	100 µl x 1	RT
Quencher Reagent	1 ml x 1	5 ml x 1	4 °C
User Manual	1 Manual	1 Manual	--

III. STORAGE AND STABILITY

All kit components are stable at -20 °C for 2 years.

IV. WORKING SOLUTION PREPARATION

1. Reaction Buffer, DMSO, Quencher reagent

All reagents must be warmed to the room temperature before use.

2. AcalephFluor®350

For label 1 mg protein size: Add 10 µl DMSO into the AcalephFluor®350 tube. Resuspend gently by withdrawing and re-dispensing the liquid once or twice using a pipette. The operation should be at room temperature, DMSO will turn to a solid at lower temperature. The dissolved reagent can be stored at -20°C for 2 weeks.

For label 5 mg protein size: Add 50 µl DMSO into the AcalephFluor®350 tube. Resuspend gently by withdrawing and re-dispensing the liquid once or twice using a pipette. The operation should be at room temperature, DMSO will turn to a solid at lower temperature. The dissolved reagent can be stored at -20°C for 2 weeks.

3. Antibody

The concentration of the antibody which need to be labeled, should be higher than 1 mg/ml. The lower concentration will affect conjugation efficiency.

Buffer Components & Conditions

Purified antibody	Yes
Antibody in ascites fluid, serum, hybridoma or tissue culture media	No
Antibody concentration	0.5-2 mg/mL
pH	6.5-8.5
Amine free buffer (e.g. MES, MOPS, HEPES, PBS)	Yes
Non-buffering salts (e.g. sodium chloride)	Yes
BSA	No
Sodium Azide	<0.1%
Chelating agents (e.g. EDTA)	Yes
Glycerol	No
Sugars	Yes
Gelatin	<0.1%
Tris	<50 mM
Glycine	No
Thiomersal / Thimerosal	No
Merthiolate	No
Proclin	No
Borate buffer	Yes
Nucleophilic components (Primary amines e.g. amino acids or ethanolamine and thiols e.g. mercaptoethanol or DTT)	No

V. ASSAY PROCEDURE

Equilibrate all materials and prepared reagents to room temperature prior to use.

1. Add 250 µl of Reaction Buffer to each 1 ml of antibody/protein to be labeled and mix gently. (Reduce the amount of Reaction Buffer at the same scale)

2. Pipette the AcalephFluor®350 into antibody/protein sample directly (1 mg antibody/protein need 10 µl AcalephFluor®350). Resuspend gently by withdrawing and re-dispensing the liquid several times using a pipette.

3. Replace cap on the vial and leave standing for 30 minutes in the dark at 37°C hot air circulating oven.

4. After incubating, add 150 µl of Quencher Reagent for each 1 ml of antibody/protein used and mix gently (Reduce the amount of Quencher Reagent at the same scale). The conjugates can be used after 5 minutes. The conjugates do not require purification.

VI. PROTEINS AND BIOMOLECULES GUIDE

Small proteins

The protocols recommend the amount of antibody to add, as this is the most common use of the kit. However, the amount can be adjusted to suit other proteins. The molar ratio of label to protein is important, as excessive amounts of the label may damage the protein or cause high backgrounds in the final assay. For example, if the protein to be labeled is 75 kDa (half the size of an antibody) the addition of 10 µg of protein would introduce twice the amount in molar terms as with 10 µg of antibody. A consequence of this may be the presence of unlabeled protein at the end of the reaction. Whether this is a concern will depend on the assay application, but the addition of less protein (5 µg in this case) would normally be a safer starting position.

Peptides/small molecules

Many peptides will have a free N-terminus i.e. with a primary amine on the first amino acid, which can participate in the reactions. In some cases, however, the N-terminus will be 'blocked' e.g. by acetyl groups, which may have been added during peptide synthesis to improve peptide stability in vivo (e.g. to prevent attack by aminopeptidases). A peptide that is both N-terminally blocked and lacks lysine residues cannot be conjugated using the Conjugation Kit.

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com