Fidabio
Labelling Kits

ALC 480 and FL 480 Fida 1 Protein Labelling Kits

Table 1. Labelling kit contents.

Reagent	Amount	Cap
Reactive dye: • FAM NHS ester (FL 480 Kit) • AF488 NHS ester (ALC 480 Kit)	3 x 100 µg	Green cap
Sodium bicarbonate	3 x 84 mg	White cap
DMSO	3 x 250 µL	Red cap
Resin for purification	5 mL	Transparent cap
Reaction tube	3 x 1 tube	Blue cap
Yellow wash tube	3 x 1 tube	
Collection tube	6 x 1 tube	
Spin columns	6 x 1	
Storage: 2 °C – 8 °C. **Do not freeze!**		

Table 2. Properties of ALC 480 and FL 480 dyes.

Catalogue no.	Dye	Molecular weight	Ex/Em maxima (nm)	ε*	Fluorescence quantum yield	CF_{260}**	CF_{280}**
420-003	ALC 480	732	495/519	71,800	0.91	0.16	0.10
410-003	FL 480	473	494/520	75,000	0.9	0.20	0.17

*Excitation coefficient in $L \cdot mol^{-1} \cdot cm^{-1}$

**Correction factor for absorption readings (A_{260}/A_{280}) at 260 nm or 280 nm.
For example, $A_{260,\,actual} = A_{260,\,observed} - (CF_{260} \times \lambda_{max})$

Introduction

The ALC 480 and FL 480 Fida 1 Protein Labelling Kits offer the ideal way to label proteins with the ALC 480 and FL 480 dyes for a FIDA experiment. The Fida 1 Protein Labelling Kits each hold everything that is required for 3 labelling reactions and purification of the formed conjugates. The reactive dyes are based on NHS coupling chemistry and are thus optimal for labelling primary amines (N-terminus and lysines). The ALC 480 is not sensitive to changes in pH (4-9), whereas FL 480 exhibits pH-dependent fluorescence and is sensitive to binding related fluorescence changes.

Before starting the labelling reaction

Allow all reagents to reach room temperature. Thereafter the steps are:

Prepare 1 M Sodium Bicarbonate Buffer

To do so, add 1 mL of deionised water to the tube containing 84 mg of sodium bicarbonate (white cap) and dissolve completely by vortexing. Store the solution at 4°C and use within one week.

Prepare the Protein Solution

Each labelling reaction requires 75 µL of protein solution. Purified protein should be at a concentration of 1–5 mg/mL in buffer. The buffer cannot contain ammonium ions or primary amines (NO Tris-HCl buffer, Ammonium acetate, Ammonium carbonate, Glycine or Histidine buffer etc.). The presence of low concentrations of sodium azide (≤ 3 mM) or thimerosal (≤ 1 mM) will not significantly affect the conjugation reaction. Glycerol concentration higher than 5 % will significantly hamper the conjugation reaction.

If the protein is in an unsuitable buffer (e.g., Tris or glycine), the buffer should be replaced by dialysis against PBS, or other means of buffer exchange e.g., Size Exclusion Chromatography.

Calculations

Perform the following calculations before beginning the conjugation reaction outlined under the “Conjugation Reaction” paragraph.

The amount of reactive dye to be used for each reaction depends upon the concentration of protein to be labelled. In the labelling procedure, a small volume of 4 mg/mL reactive dye stock solution (prepared in step 1.3 re the Experimental Protocol below) is added to 75 µL of protein solution. Calculate the volume of the 4 mg/mL reactive dye stock solution to be added (in step 1.4 re the Experimental Protocol below) using the following formula:

$$\text{µL of reactive dye stock solution} = \frac{{}^{mg}/_{mL}\ protein \times V\ \mu L}{MW_{protein} \times C\ {}^{mg}/_{mL}} \times MW \times MR$$

- V is the volume of protein solution, V = 75 µL

- MW is the molecular weight of the reactive dye, MW_{ALC480} =732, MW_{FL480} =473
- C is the concentration of the dye DMSO solution, C=4 mg/ml.
- For most whole IgGs, $MW_{protein}$=145,000
- MR is the molar ratio of dye to protein in the reaction mixture. For IgG labelling reactions, we recommend an MR of 5. For other proteins, the MR usually ranges from 5 to 10. You can choose to perform two reactions at different MRs to better ensure optimal labelling.

Experimental protocol

1. Conjugation reaction

1.1 Transfer 75 µL of protein solution to a 500 µL reaction tube (Blue cap).

1.2 Add 7.5 µL of freshly prepared 1 M sodium bicarbonate solution to the protein-containing reaction tube (1/10 of the protein sample solution).

1.3 Prepare the 4 mg/mL reactive dye stock solution. Immediately before starting the reaction, add 25 µL of DMSO (Red cap) to the vial of reactive dye (Green cap, 100 µg dry dye). Pipet up and down to completely dissolve the contents of the vial.

1.4 Add the appropriate amount of dye (see Calculations). Discard the remaining reactive dye stock solution.

1.5 The reaction mixture is incubated at room temperature for at least 30 minutes, protected from light.

2. Labelled protein purification

2.1 Shake the bottle with resin to homogenise the resin. Mix the resin by pipetting up and down with an appropriate pipette i.e., 1,000 µL and transfer 400 µL of the resin to the spin column.

2.2 Snap off the bottom stopper and place the spin column in a yellow wash tube.

2.3 Spin the column for 60 seconds at 0.8 x 1,000 RCF. NOTE! If you use a fixed angle microcentrifuge, put a mark at the top of the spin column to keep track of the position of the column. Discard the wash tube. Do not allow the resin bed to dry out. Process the sample (2.4 and 2.5) within the next few minutes.

2.4 Place the spin column in a collection tube (Colourless Eppendorf tube) carefully (drop-wise) apply ≈ 75 µL of the sample directly to the centre of the resin bed without touching the resin bed or the sides of the column since this can reduce the efficiency of the purification.

2.5 Spin the column for 120 seconds at 0.8 x 1,000 RCF. Collect the labelled protein in the flow-through.

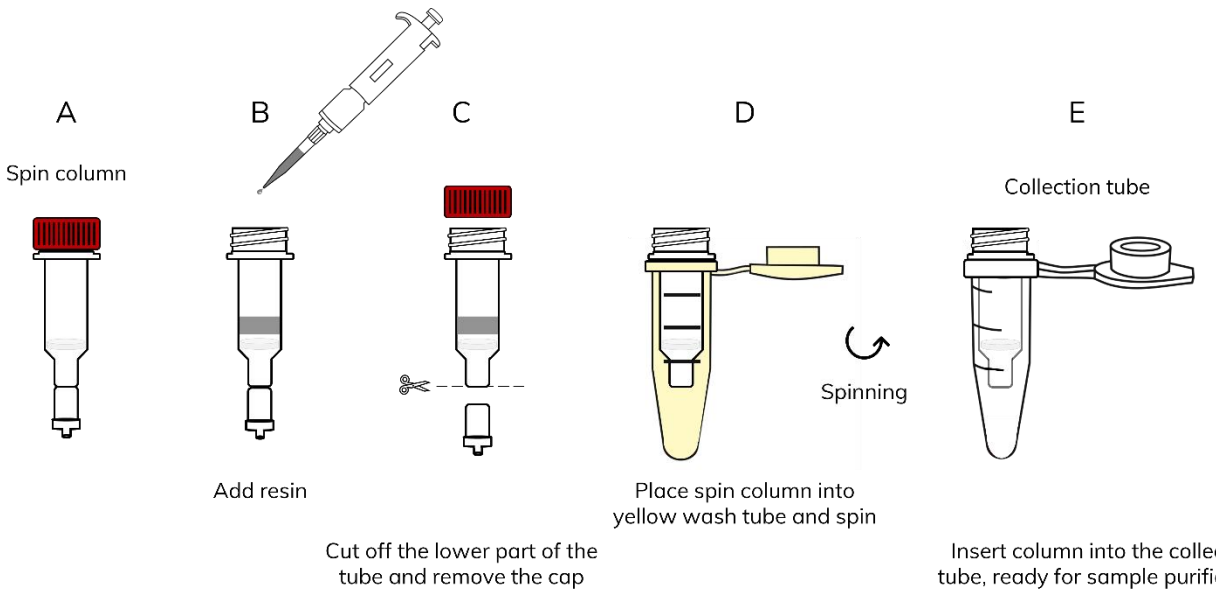

Figure 1. A. Spin column, **B-D.** Buffer removal from resin, **C.** Assembly of resin tube with collection tube.

3. Degree of Labelling (DOL) determination

Note: *Before determining the Degree of Labelling (DOL) perform a test on the Fida 1 instrument to determine the degree of free fluorophore. If the degree of free fluorophore is high, then perform another dye removal (purification).*

Measure the absorbance and the labelled protein at both 280 nM (A_{280}) and 495 nm (A_{495}). Calculate the protein concentration using this equation:

$$\frac{C_{protein} mg}{mL} = \frac{[A_{280} - (A_{495}\ x\ \mathrm{CF280})]\ x\ dilution\ factor}{\varepsilon_{protein}} x\ MW_{protein}$$

- CF_{280} is the correction factor of the dye at 280 nm, $CF_{280,\ ALC480}$=0.10, $CF_{280,\ FL480}$=0.17
- $\varepsilon_{protein}$ is the molar extinction coefficient of the protein at 280 nm

The dilution factor corresponds to how much you diluted the protein before measuring the absorbance.

Calculate the Degree of Labelling with this equation:

$$Dye\ molecules\ per\ protein = \frac{A_{495}\ x\ dilution\ factor}{\varepsilon dye\ x\ C_{protein}}\ x\ MW_{protein}$$

- ε_{dye} is the extinction molar coefficient of the conjugated dye, $\varepsilon_{dye,\ ALC480}$=71,800, $\varepsilon_{dye,\ FL480}$=75,000

Storing the conjugates

Store the conjugates at 2 °C–8 °C, protected from light. If the final concentration of purified protein conjugate is less than 1 mg/mL, add BSA or other stabilizing protein at 1–10 mg/mL. In the presence of 2 mM sodium azide, the conjugate should be stable at 2 °C-8 °C for several months. For longer storage, divide the conjugate into small aliquots and freeze at ≤ –20 °C. Avoid repeated freezing and thawing. Protect from light.

Products

Catalogue no.	Product name
420-003	ALC 480 Fida 1 Protein Labelling Kit (3 reactions, 100 µg each)
410-003	FL 480 Fida 1 Protein Labelling Kit (3 reactions, 100 µg each)

Buyer notice

Support

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Safety Data Sheets (SDS)

FAM NHS Ester, 6-isomer
AF488 NHS Ester
DMSO
Sodium Bicarbonate
Resin

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