Attaching Fluorescein to a single cysteine in proteins

Labeling buffer (45 ml):

10 mM K-Hepes (pH 7.0)	1M stock	0.45 mL
100 mM KCl	3M stock	1.5 mL
20 μM GDP*	100 mM stock	9.0 μL
Water		43.0 mL

Note: GDP is needed only for EF-Tu or RF3

5 mM Iodoacetamidofluorescein (FW =515):
Freshly dissolve 1.03 mg in 400 μL of Dimethylformamide (DMF). Do not store, discard unused reagent.

250 μM β-Mercaptoethanol:
Mix 2 μl (14.4 M) mercaptoethanol stock with 498 μl of water = 57.6 mM stock.
Mix 2 μl (57.6 mM stock) with 458 μl of water = 250 μM stock.

Protein is stored in Labeling Buffer: 10 mM HEPES-KOH (pH 7.0), 100mM KCl, 20 μM GDP*.

Labeling Reaction:

125 µM Protein	100 μM final conc.	160 μl
250 μM β-mercaptoethanol	25 μM final conc.	20 μl
5 mM Iodoacetamidofluorescein	500 μM final conc.	20 μl

Add Labeling buffer to 200 μl final volume.

1. Mix EF-G proteins (100 µM) and limiting β-mercaptoethanol (β-ME; 25 µM) first and incubate at room temperature for 1 hour to reduce the cysteines. Then add the Iodoacetamidofluorescein to start the labeling reaction.

2. Incubate reaction in the dark (20^{o}C, overnight).

3. Stop reaction by adding 1.4 µl of 14.4 M β-ME (final conc. is 100 mM).

4. Purify EF-G by passing through a Sephadex G-25 or BioRad P2 column (make sure that the molecular weight cut-off is appropriate for the column).

5. Analyze a small amount by SDS-PAGE. The labeled protein will fluoresce under UV light.

6. Dialyze against storage buffer, and store at -80^{o}C in small aliquots.

The ratio *R* of FL attached to EF-G varied between 0.6 and 0.8, as determined by the protein's absorbance of light at 280 and 492 nm, and the equation:

$R = (A492 \times \varepsilon EF\text{-}G)/(A280 \times \varepsilon FL)$,

where εFL = 78,000 M-1cm-1 and $\varepsilon EF\text{-}G$ = 64,282 M-1cm-1.

NOTES:

-The dye (FL) is not stable after dissolving in DMF. So dissolve only small quantities by weighing few specks on weighing paper and adding DMF to appropriate volume.

-It may be necessary to purify the protein by gel filtration after labeling.