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Protein Labeling Kits

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Detailed protocol is available at **www.lifetechnologies.com/manuals**.

WARNING! For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Material		Amount	Storage*
Reactive dye (solid) + Stir bar	(Component A)	3 vials	• 2–8°C • Protect from light
Sodium bicarbonate (solid)	(Component B)	84 mg	
Purification resin (slurry)	(Component C)	~25 mL	
10X Elution buffer (liquid)	(Component D)	~25 mL	
Purification columns, column funnels, foam holders, collection tubes		3 each	

*The kit can be stored under the conditions listed. For optimal storage conditions of individual components, refer to the labels on the vials or bags. **Do not freeze Components C and D.**

Prepare protein

Protein purity: For optimal labeling efficiency, the protein must be free of ammonium ions, imidazole, primary amines (e.g. Tris, glycine), and other proteins/peptides (e.g. BSA, gelatin, serum). Purify the protein as needed.

Protein concentration/volume: For efficient labeling, protein concentration should be at 2 mg/mL in 0.5 mL of 1X PBS (pH 7.2–8.0) or 0.1 M sodium bicarbonate buffer (pH 8.0–8.3).

Label protein

1. **Prepare 1 M solution of sodium bicarbonate buffer:** Add 1 mL of distilled water to the entire vial of Component B (84 mg sodium bicarbonate). Mix to dissolve.
2. **Dilute protein:** If the protein concentration is >2 mg/mL, dilute the protein to 2 mg/mL (e.g. 1 mg of protein in 0.5 mL of 1X PBS or 0.1 M sodium bicarbonate buffer).
3. **Adjust pH:** Add 50 µL of 1 M sodium bicarbonate buffer (from step 1) to 0.5 mL of protein in 1X PBS; final pH should be ~7.5–8.3. Skip this step if the protein is already in sodium bicarbonate buffer (pH 8.0–8.3) or PBS (pH 8.0).
4. **Prepare labeling reaction mixture:** Add the protein solution (from step 3) to 1 vial of reactive dye (Component A). Cap the vial and mix gently by inversion or by pipetting up and down. Stir the reaction mixture. Do not froth the solution.
5. **Incubate labeling reaction:** Stir the reaction mixture at slow speed on a magnetic stirrer for 1 hour at room temperature. Do not froth the solution.

   Note: Because the preparation of the purification column takes ~15 minutes, you may wish to begin pouring the column (see **Purify labeled protein**, next page) during the labeling reaction.

Purify labeled protein

6. **Prepare purification column/resin:** Assemble the purification column as shown in Figure 1.

   Add purification resin (Component C) up to ~3 cm from top. Check buffer flow-through with 1X PBS.

   If buffer flow stalls, remove the resin, clear the frit, and repack the column with resin/buffer. Allow the excess buffer to drain before loading the labeled protein.

7. **Prepare 1X elution buffer:** Dilute 10X elution buffer (Component D) 10-fold in distilled water at room temperature. Typically, less than 10 mL will be required for each purification.

8. **Purify protein:** Remove the funnel. Load the reaction mixture (from step 5) onto the top of the resin in the column; allow the mixture to settle into resin.

   Elute the protein with 1X elution buffer (from step 7) or 1X PBS. Collect and retain all fractions.

9. **Calculate DOL (degree of labeling) and store labeled protein:** Refer to the detailed protocol available at **www.lifetechnologies.com/manuals**.

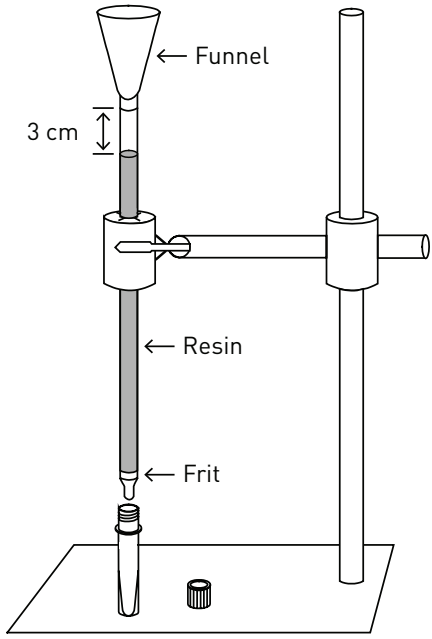

Figure 1

Documentation and Support

For additional product and technical information, such as Safety Data Sheets (SDS), Certificates of Analysis, etc., visit our website at **www.lifetechnologies.com**. For further assistance, email our Technical Support team at **techsupport@lifetech.com**.

Limited Product Warranty

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