IRDye® 680RD Protein Labeling Kit - Microscale

Labeling Instructions

Please read the pack insert completely before using the kit. This card is intended as a guide for use in the lab and does not contain all the information that is included in the pack insert.

1. Determine the volume of dye needed for the labeling reaction based on the molecular weight of the protein. Use the table for MWs specifically listed in the pack insert; other values may be interpolated. Alternatively, use the equation for any MW within the range 15 – 200 kDa: Dye volume (µL) = 100 / MW.
2. Prepare 100 µg of protein in 100 µL of phosphate buffer; protein concentration must be 1 ± 0.1 mg/mL.
3. Raise the pH of the protein solution to 8.5 by adding 1 M potassium phosphate buffer provided in kit; 10 µL works for 100 µL of protein in PBS.
4. Warm dye and protein solutions to room temperature; protect dye from light.
5. Dissolve 1 tube of dye in 25 µL ultrapure water provided in kit. Vortex until dye is completely dissolved.
6. Add the appropriate amount of dye to the protein solution. Mix thoroughly by inversion. Incubate 2 hours at ambient temperature; protect from light.
7. Purify the conjugate using the spin columns supplied in kit. See the opposite side of this Quick Card for detailed spin column instructions.

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Quick Card

Separation of Conjugate from Unreacted Dye Using Zeba™ Spin Desalting Columns

1. Remove the bottom closure from the column and loosen but do not remove cap.
2. Place column in a 1.5 – 2 mL microcentrifuge collection tube.
3. Centrifuge column at 1,500 x *g* for 1 minute to remove storage solution.
4. Wash column 3 times with 300 µL of 1X PBS; centrifuge at 1,500 x *g* for 1 minute; discard wash solution.

   Note: *Resin will appear compacted and dry after each wash and centrifugation.*
5. Place column in a new collection tube, remove cap and slowly apply the entire sample (approximately 100 µL) to the center of the compact resin bed.
6. Centrifuge at 1,500 x *g* for 2 minutes to collect the sample. Discard the column after use.

   Note: *Never reuse the Zeba™ Spin Desalting Columns.*

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