SpectraDye Labeling Kit Easy Protocol

Directions for labeling various amounts of antibody

Ab Starting Material (µg)	Ab Volume (µL)	Antibody Labeling Buffer (µL)	Dye Solution (µL)	Quenching Solution (µL)	Neutralization Buffer (µL)
1	10 (0.1 mg/mL)	2.5	1.2	3 (Diluted 1:10 with H_20 prior to use)	8
10	10 (1 mg/mL)	2.5	1.2	3 (Diluted 1:10 with H_20 prior to use)	8
100	100 (1 mg/mL)	25	12	3	8
1000	1000 (1 mg/mL)	250	120	30	80

Directions for labeling 100 µg of antibody

Important: Equilibrate all reagents to ambient temperature before use.

Note: **For optimal performance the antibody to be labeled should be in a suitable primary amine-free buffer such as PBS, MES, MOPS or HEPES.** If your antibody is in an inappropriate buffer we recommend exchanging the buffer to 1X PBS with G-25 Desalting Spin Columns (Advansta Cat# L-07131-005).

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1. Suspend the antibody of interest in PBS to create a 1 mg/ml concentration.
2. Add 25 µl Antibody Labeling Buffer to 100 µl antibody solution from step 1.
3. Mix well by pipetting up and down a few times.
4. Add 12 µl of Dye Solution to the reaction and mix by pipetting.
5. Incubate for 30 min at room temperature.
6. Add 3 µl Quenching Solution to the reaction mixture.
7. Incubate for 5 min at room temperature.
8. Add 8 µl Neutralization Buffer to the reaction mixture.
9. Antibody is now labeled and ready to use.