Quick Protocol, continued

SpectraDye Antibody Labeling protocol (user supplied dye):

1. Equilibrate all reagents to ambient temperature before use.
2. Centrifuge all tubes to collect contents prior to use.
3. Prepare dye solution at 0.42 mM in DMSO. (**Note:** It is very important to use the high quality DMSO supplied in the kit).
4. Add 25 µL of SpectraDye Ab Labeling Buffer to each 100 µL of Ab solution at 0.1-3 mg/mL and mix well by pipetting up and down a few times.
5. Add 12 µL of dye solution for every 100 µL of Ab solution used and mix well by pipetting up and down a few times.
6. Incubate at ambient temperature for 30 minutes.

For immediate use of labeled antibody and to avoid potential background resulting from unhydrolyzed dyes, we recommend the follow steps after labeling:

7. Add 3 µL Quenching Solution for every 100 µL of Ab solution used, mix well and incubate at ambient temperature for 5 minutes.
8. Add 8 µL Neutralization Buffer for every 100 µL of Ab solution used.
9. Antibodies are now labeled and ready to use.

For More Information

visit **www.advansta.com/products/SpectraDye** or go directly to the web-page by scanning the QR-code with your favorite bar-code scanner app on your smart phone.

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SpectraDye™ Antibody Labeling Kits

Fluorescently label antibodies in one easy step

For Catalog Numbers

K-11054-010	SpectraDye Antibody Labeling Kit-350, 1 kit
K-11055-010	SpectraDye Antibody Labeling Kit-490, 1 kit
K-11056-010	SpectraDye Antibody Labeling Kit-550, 1 kit
K-11057-010	SpectraDye Antibody Labeling Kit-650, 1 kit
K-11058-010	SpectraDye Antibody Labeling Kit-IR700, 1 kit
K-11059-010	SpectraDye Antibody Labeling Kit-IR800, 1 kit
K-11060-010	SpectraDye Antibody Labeling Kit, user-supplied dye, 1 kit

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SpectraDye™ Antibody Labeling Kits

Description

SpectraDye Antibody Labeling Kits produce covalently labeled, fluorescent primary antibodies in a single, 30-minute step. SpectraDye-labeled antibodies are compatible with all immunofluroescence methods. Use the same antibody across multiple platforms for increased reliability and reproducibility.

Storage Information

SpectraDye Dye Solution and all kit components should be stored at 4°C. Each SpectraDye Antibody Labeling kit includes the following: Antibody Labeling Buffer 250 µL, SpectraDye Dye Solution 125 µL*, Quenching Solution 30 µL, and Neutralization Buffer 80 µL. Kit K-11060-010 also includes 1mL DMSO.

* Trial kit includes 13 µL of SpectraDye Dye Solution. Kit K-11060-010, user-supplied dye, does not include SpectraDye Dye Solution.

Warnings and Precautions

- SpectraDye Antibody Labeling Kits are for research use only.
- Always wear gloves when handling reagents.
- Refer to MSDS for additional safety information.
- The product is guaranteed to be free of manufacturer defects, and to function as described when the enclosed protocol is followed by properly trained personnel.

Quick Protocol

1. **For optimal performance the antibody to be labeled should be in a suitable primary amine-free buffer such as PBS, MES, MOPS or HEPES.**
   - Avoid use of the following buffer components because they will decrease labeling efficiency:
     - Nucleophilic components such as Tris, glycine, amino acids and ethanolamine
     - Thiols and reducing agents such as DTT, mercaptoethanol and TCEP
     - Stabilizers such as glycerol and albumin
   - If your antibody is in an inappropriate buffer we recommend exchanging the buffer to 1X PBS with G-25 Desalting Spin Columns (Cat# L-07131-005)
2. Equilibrate all reagents to ambient temperature before use.
3. Centrifuge all tubes to collect contents prior to use.
4. Add 25 µL of Ab Labeling Buffer to 100 µL of Ab solution at 0.1-3 mg/mL and mix well by pipetting up and down a few times.
5. Add 12 µL of SpectraDye Dye Solution for every 100 µL of Ab solution used and mix well by pipetting up and down a few times.
6. Incubate at ambient temperature for 30 minutes.

For immediate use of labeled antibody and to avoid potential background resulting from unhydrolyzed dyes, we recommend the follow steps after labeling:

7. Add 3 µL Quenching Solution for every 100 µL of Ab solution used, mix well and incubate at ambient temperature for 5 minutes.
8. Add 8 µL Neutralization Buffer for every 100 µL of Ab solution used.
9. Antibodies are now labeled and ready to use.