INSTRUCTIONS

DyLight® Dye-Labeled Phosphine

88907 88910 88911

2201.3

Number	Description
88907	**DyLight 488-Phosphine**, 1mg Molecular Weight: 1088.01
88910	**DyLight 550-Phosphine**, 1mg Molecular Weight: 1331.37
88911	**DyLight 650-Phosphine**, 1mg Molecular Weight: 1357.41

Storage: Upon receipt store at -20°C in foil pouch with desiccant. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific DyLight Dye-Labeled Phosphine Reagents are azide-reactive fluorescent dyes. DyLight Dyes are more intense than Alexa Fluor® or Cy® Dyes in many applications, match the output wavelengths of common fluorescence instrumentation, and fluoresce over a broad pH range.

The phosphine-activated DyLight Dyes are ideal for labeling azide-containing molecules metabolically incorporated into cells. The phosphine group reacts with an azide to produce an aza-ylide intermediate that is trapped to form a stable, covalent amide bond (Figure 1), which is also referred to as the Staudinger reaction.[1] Because azides are absent from biological systems, there is minimal background labeling of cells or lysates.[2]

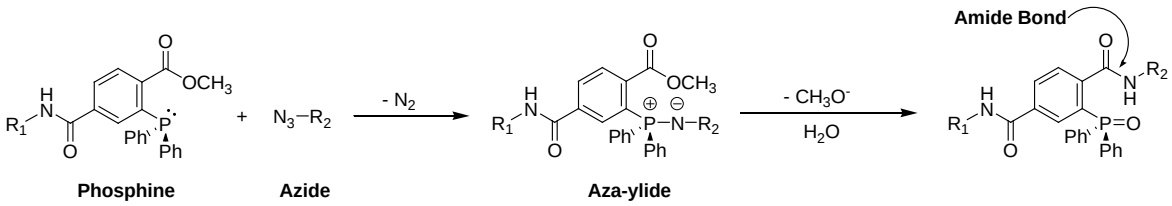

Figure 1. Reaction scheme of phosphines and azides, which is also referred to as the Staudinger reaction.

Important Product Information

- Avoid reducing agents in reaction buffers, which may interfere with target azide stability.
- Reactions between phosphines and azides are more efficient at high concentrations and temperatures (23-37°C). Typical reaction times are from 30 minutes to 3 hours; however, longer incubation times may improve reaction efficiency.
- Use the following fluorescent imagers: DyLight 488 Dye: Green (526) laser, DyLight 550 Dye: Green (532) laser and DyLight 650 Dye: Red (633) laser. See the table below for more properties of the fluorescent dyes.

DyLight Fluor	Ex/Em*	ε†	Spectrally Similar Dyes
488	493/518	70,000	Alexa Fluor 488, Cy2
550	562/576	150,000	Alexa Fluor 555, Cy3
650	646/674	250,000	Alexa Fluor 647, Cy5

* Excitation and emission maxima in nanometers
†Molar extinction coefficient (M^{-1} cm^{-1})

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Reagent Preparation

Note: Dissolve the phosphine reagent in a dry water-miscible organic solvent, such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF), before diluting in final reaction buffer. Dissolve the reagent on the tube wall, and pipette the solution up and down to completely dissolve. Store stock solutions at -20°C for up to 4 weeks. Do not store reagent in aqueous solutions. Avoid multiple freeze-thaw cycles.

Prepare 10mM of the phosphine reagent by dissolving 1mg with organic solvent as follows:

- DyLight 488-Phosphine, add 92µL of solvent
- DyLight 550-Phosphine, add 75.1µL of solvent
- DyLight 650-Phosphine, add 73.7µL of solvent

Procedure for Azide-containing Protein or Cell Lysate Labeling

A. Additional Materials Required

- Water-miscible organic solvent such as DMSO or DMF
- Phosphate-buffered saline (PBS) or other buffer at pH 6-8
- Thermo Scientific Dye Removal Columns (Product No. 22858)

B. Azide Protein or Lysate Staining

1. Prepare azide-containing sample in PBS or other suitable aqueous buffer.

   Note: Proteins and lysates may be conjugated with NHS-azide reagents (see the Related Thermo Scientific Products Section).

2. Add the 10mM phosphine stock solution to a final concentration of 50-200µM to the azide-containing sample in PBS. If the azide-containing protein is ≥ 5mg/mL, use a 10-fold molar excess of the phosphine reagent; for samples < 5mg/mL, use a 20-fold molar excess.
3. Incubate the reaction at 37°C for 2-4 hours. Reactions may be incubated at room temperature but will require a longer incubation (16-24 hours).
4. Remove non-reacted phosphine reagent using the Dye Removal Columns.

Procedure for Cell Labeling

Note: Individual protocols are included below for live-cell and fixed-cell labeling.

Additional Materials Required

- Cells maintained in an appropriate cell culture medium
- DMSO or DMF
- Hank's Balanced Buffered Saline (HBSS)
- Fetal bovine serum (FBS)
- Phosphate-buffered saline (PBS) or other buffer at pH 6-8
- Formaldehyde 16% (w/v), Methanol-free (Product No. 28906 or 28908)
- Thermo Scientific Blocker BSA in PBS (10X) (Product No. 37525)
- Tween®-20 Detergent (Thermo Scientific Surfact-Amps 20, Product No. 28320)
- Hoechst 33342 solution (Thermo Scientific Hoechst 33342 Solution, Product No. 62249)

Live-cell Labeling

1. Incubate cells for 2-3 days in culture medium containing 20-40µM of azide-sugar.
2. Rinse cells with HBSS containing 2% FBS. Dilute the 10mM phosphine stock solution to 50-200µM in HBSS with 2% FBS. Add the phosphine solution to the cells and incubate at 37°C for 3 hours.

   Note: Optimal incubation time with the phosphine solution ranges from 30 minutes to 4 hours.
3. Prepare 4% formaldehyde by diluting the 16% formaldehyde 1:4 with PBS.
4. To remove excess phosphine reagent, rinse cells twice with HBSS with 2% FBS.
5. Add 4% formaldehyde to cells for 15 minutes at room temperature.
6. To remove formaldehyde, rinse cells twice with PBS.
7. Stain cells with Hoechst 33342 in PBS for 10-30 minutes.
8. Wash cells two times with PBS.
9. Observe cells using an appropriate imager.

Fixed-cell Labeling

1. Incubate cells with an azide containing metabolite in cell culture media.
2. Just before use, prepare 4% formaldehyde by diluting the 16% formaldehyde 1:4 with PBS.
3. Rinse cells twice with PBS and add 4% formaldehyde to cells for 15 minutes at room temperature.
4. Rinse cells twice with PBS to remove formaldehyde.
5. Block cells with 1X BSA in PBS for 30 minutes at room temperature.
6. Dilute the 10mM phosphine solution to 50-200µM with 1X BSA in PBS. Add the phosphine solution to cells and incubate at 37°C for 1-3 hours.
7. To remove excess phosphine reagent, rinse cells three times with 0.5% Tween-20 Detergent in PBS.
8. Stain cells with Hoechst 33342 in PBS for 10-30 minutes.
9. Wash cells two times with PBS.
10. Observe cells using an appropriate imager.

Troubleshooting

Problem	Possible Cause	Solution
Low staining efficiency	Suboptimal reaction conditions	Optimize conjugation conditions by altering molar excess of phosphine to azide
		Perform conjugation reactions at 37°C
		Increase incubation time
High background staining	Excess dye not removed	Repeat dye removal column chromatography for azido proteins or lysates
		Increase incubation time in 5% BSA in PBS
		Increase washes with 0.5% Tween-20 in PBS

Related Thermo Scientific Products

88901	**Biotin-PEG$_3$-Phosphine**, 10mg
88903	**GlcNAz** (*N*-azidoacetylglucosamine, tetraacylated), 5mg
88904	**ManNAz** (*N*-azidoacetylmannosamine, tetraacylated), 5mg
88905	**GalNAz** (*N*-azidoacetylgalactosamine, tetraaceylated), 5mg
26130	**NHS-PEG$_4$-Azide,** 100mg
26131	**NHS-PEG$_{12}$-Azide,** 100mg
88902	**NHS-Azide**, 10mg
88900	**NHS-Phosphine**, 10mg
88906	**Sulfo-NHS-Phosphine**, 10mg
28372	**BupH™ Phosphate Buffered Saline Pack,** 40 packs
28906	**Formaldehyde 16% (w/v), Methanol-free,** 10 × 1mL ampule
28908	**Formaldehyde 16% (w/v), Methanol-free,** 10 × 10mL ampule
37525	**10X Blocker BSA in PBS,** 200mL
28320	S**urfact-Amps 20,** 6 × 10mL
22858	**Dye Removal Columns**
62249	**Hoechst 33342 Solution,** 5mL

References

1. Saxon, E. and Bertozzi, C. (2000). Cell surface engineering by a modified Staudinger reaction. *Science* **287:**2007-10.
2. Agard, N., *et al.* (2006). A comparative study of bioorthogonal reactions with azides. *ACS Chemical Biology* **1(10):**644-8.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.