

# Perform labeling and other reactions in Slide-A-Lyzer<sup>®</sup> Dialysis Cassettes

TR0014.2

## Introduction

Biotinylation and other labeling reactions generally involve using a 10- to 20-fold molar excess of reagent to protein target. This excess reagent ensures that all or most of the target functional groups on the protein are labeled, but it also necessitates that the non-reacted reagent and reaction by-products subsequently be removed by dialysis or desalting. Having to process a completed labeling reaction by transferring it into dialysis tubing or onto a desalting column for clean up increases the possibility that sample loss will occur, especially for small scale reactions with dilute protein.

Thermo Scientific Slide-A-Lyzer Dialysis Cassettes and MINI Units may be used not only for dialysis following a labeling reaction but also as the labeling reaction vessel. The Cassettes are available in several different volume formats (0.1-0.5 ml, 0.5-3 ml, 3-12 ml) and molecular weight cut-off membranes (2, 3.5, 7, 10 and 20K). The MINI Units are for efficient dialysis of 10-100  $\mu$ l samples (see our website for a complete listing of Slide-A-Lyzer Dialysis Products).

Generally, aqueous buffered labeling reactions may be performed as follows:

1. Combine labeling reagent and protein in reaction buffer.
2. Inject reaction mixture into an appropriately sized Slide-A-Lyzer Dialysis Cassette.
3. Use float buoys to make a stand and incubate cassette on bench top or rocking platform for 30-60 minutes.
4. Transfer cassette with float buoy to beaker containing dialysate.
5. Dialyze until non-reacted labeling reagent and by-products are removed (6-18 hours with several changes of the buffer).
6. Retrieve labeled protein from the dialysis cassette.

## Important Tips Regarding this Method

- Dialysis is appropriate only for separating large, labeled products from small reagent by-products. Choose a dialysis membrane with a molecular weight cut-off (MWCO) that is both smaller than the protein or other molecule being labeled and significantly greater than the size of the non-reacted labeling reagent. Be aware that some labeling reagents, especially certain dyes, form aggregates that behave as large molecules.
- Use this method only for reactions in which all products and by-products are soluble. It will be difficult to recover a sample that contains precipitate.
- Avoid reaction mixtures involving solvents to which the regenerated cellulose has poor chemical resistance. For example, dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF) are often used to dissolve labeling reagents and then maintained at 10-20% concentration in the final reaction volume. Dialysis membrane has only limited resistance to these organic solvents and will probably fail unless the reaction time before dialysis is minimized. Alcohols such as methanol and ethanol are generally compatible with the dialysis membrane; however, care must be taken to control evaporation of these more volatile solvents (see next tip).
- Take measures to avoid excessive evaporation from the cassette during the labeling reaction. For reaction times greater than 30 minutes, consider sealing the cassette in a sealable plastic bag or wrapping it in plastic wrap.
- If constant mixing of the reaction is required, leave an air bubble in the dialysis cassette upon injection of sample and then secure the cassette on a rocker or shaker platform for the duration of the reaction time.
- Avoid this method for reactions that affect the membrane material. For example, sodium meta-periodate, which is commonly used to oxidize carbohydrate groups to create reactive aldehydes, will oxidize the cellulose membrane. Likewise, enzyme reactions involving cellulase will result in digestion of the membrane.

Current versions of product instructions are available at [www.thermo.com/pierce](http://www.thermo.com/pierce). For a faxed copy, call 800-874-3723 or contact your local distributor.

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