

Conjugation of Affibody[®] Molecules

MATERIALS AND BUFFERS REQUIRED

PBS: 2.68 mM KCl, 1.47 mM KH₂PO₄, 137 mM NaCl, 8.1 mM Na₂HPO₄, pH 7.4

Desalting column: NAP5-column (GE Healthcare cat no 17-0853-01)

Dithiothreitol (DTT) solution

Fluorescent dye: Any thiol reactive fluorescent dye, e.g. Oregon Green[®] 488 maleimide (Molecular Probes O6034)

Desalting spin columns: (Pierce cat no 89849)

INTRODUCTION

The Affibody[®] molecule is delivered with a unique C-terminal cysteine that is easily conjugated with any fluorescent dye of choice. Bright and strong staining has been obtained with the Oregon Green[®] dye, one of many dyes that are available as a thiol reactive derivative.

The Affibody[®] molecule is conjugated as described by the manufacturer. However, the Affibody[®] molecules are partially dimerized due to S-S bridges formed by the C-terminal cysteine and reduction of the Affibody[®] molecule immediately prior to conjugation is therefore an absolute necessity. A brief protocol for reduction of the C-terminal cysteine is found below.

REDUCTION OF THE AFFIBODY[®] MOLECULE

1. Dissolve the lyophilized Affibody[®] molecule in PBS to obtain a final concentration of 1 mg/ml.
2. Add DTT to a final concentration of 20 mM at >pH 7.5.
3. Incubate at room temperature for 2 hours.
4. Remove excess DTT by passage through a NAP5-column. The Affibody[®] molecule will re-dimerize quickly and dialysis is therefore not recommended.
5. Immediately after step 4 above, add the conjugate at the recommended molar excess and follow the protocol from the fluorescent dye manufacturer.
6. After completed conjugation and dialysis, we strongly recommend an extra desalting step using protein desalting spin columns to remove all remaining free fluorescent dye.

LIMITATIONS

Warranty: Affibody[®] products are warranted to meet stated product specifications and to confirm to label descriptions when used and stored properly. Unless otherwise stated, this warranty is limited to one year from date of sales for products used, handled and stored according to Affibody AB's instructions. Affibody AB's sole liability is limited to replacement of the product or refund of the purchase price. Affibody[®] products are supplied for research use only. They are not intended for medicinal, diagnostic or therapeutic use. Affibody[®] products may not be resold, modified for resale or used to manufacture commercial products without prior written approval from Affibody AB.

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