info@chromotek.com
1/27/2021

Application note

Maleimide chemistry labeling protocol of Nanobodies

Content

1. Introduction

This protocol provides recommendations for the site-directed labeling of ChromoTek Nanobodies containing 2 ectopic cysteines with thiol-reactive fluorescent dyes by maleimide chemistry.

2. General considerations and recommendations

- Each fluorescent dye is different and can influence the Nanobody to a different extent. The conditions for labeling must be established individually for each dye.
- Remember that Nanobodies are only 1/10 the size of an antibody when antibody labeling kits are used.
- Many fluorescent dyes have a hydrophobic structure. The conjugation of hydrophobic dyes to Nanobodies can affect the solubility of the Nanobody.

3. Preparation of dye

- Follow the dye manufacturer's protocol.
- Freshly prepare the dye stock solution immediately before starting the labeling reaction. Functional groups lose their reactivity during storage.
- Adjust the molar excess of the dye according to the dye manufacturer's recommendations. Use at least 2 equivalents of dye per Nanobody (corresponds to 1 equivalent of dye per

cysteine) to ensure complete labeling of both cysteines. A greater excess of the dye may be needed depending on the reactivity of the dye.

- Dyes are dissolved in organic solvents. Note that organic solvents can affect the stability and can facilitate precipitation of the Nanobody.

4. Preparation of VHH

- Centrifuge material before use (20,000x g, 15 min, +4°C).
- Nanobodies are stored in HEPES buffer (10 mM HEPES pH 7.0, 500 mM NaCl, 1 mM TCEP), which is compatible with many dyes and labeling protocols. An additional buffer exchange step is not necessary.
- Note that the labeling buffer can influence the labeling efficiency.

5. Conjugation reaction

- Mix the diluted dye with the Nanobody.
- Place the tube on ice and incubate for 1-2 h.
- Optional: Overlay the labeling reaction with argon.

6. Removal of unbound dye

- Centrifuge the solution after the labeling reaction is completed (20,000x g, 15 min, +4°C) and continue working with the supernatant.
- Separate unbound dye from the labelled Nanobody by one of the following options or by a combination thereof:
    - Size exclusion column (length: >30 cm)
    - Dialysis (molecular weight cut off: 3.5 kDa)
    - Spin column (molecular weight cut off: 7 kDa)
    - Desalting column

7. Storage

- Aliquot the labelled Nanobody and store at -20°C. Avoid freeze-thaw cycles. Protect from light.
- Add 0.1% sodium azide for long-term storage to prevent bacterial contamination.