Monolith X Protocol MOX-P-106

Size exclusion chromatography of membrane proteins to remove free dye captured by micelles

Detergents are often used to solubilize membrane proteins prior to biochemical investigation. To measure binding affinities using temperature-related intensity change (TRIC) or Spectral Shift, the first step is to label one of the binding partners. If this molecule is a membrane protein solubilized in detergent at a concentration above the CMC, it may be necessary to adapt the labeling protocol. Free detergent micelles can capture excess free dye molecules resulting in 1) a lower dye concentration available in the labeling reaction and 2) the presence of micelle-captured free dye during the binding affinity measurement. This can lead to decreased labeling efficiency and signal quality.

This protocol was tested for NHS, Maleimide and tris-NTA labeling, but can be adjusted for other labeling strategies.

Membrane proteins | Labeling | Purification | Size Exclusion Chromatography | MOX-P-106

A. Applicable Labeling Kits

Protein Labeling Kit RED – NHS 2nd Generation (MO-L011, NanoTemper Technologies GmbH)

Protein Labeling Kit RED – MALEIMIDE 2nd Generation (MO-L014, NanoTemper Technologies GmbH)

NT-L021 Spectral Shift Optimized Protein Labeling Kit Lysine-Reactive

NT-L024 Manual Spectral Shift Optimized Protein Labeling Kit Cysteine-Reactive

B. Material:

Superdex 200 Increase 10/300 GL column (Cytiva)

ÄKTA pure system (Cytiva)

100 µL of labeling reaction according to the labelling kit protocol

C. Protocol

1. Prepare and filter 500 mL of chosen assay buffer including detergent.

All steps are performed with a 1 mL/min flow rate at room temperature

2. Connect the SEC column and wash it with two column volumes of water, followed by two column volumes of buffer.
3. Label your membrane protein according to standard labelling manual up until and including the step of mixing and incubating the target molecule and dye.
4. Inject the labelled membrane protein (100 µL, 10 µM).
5. Set ÄKTA absorbance detectors to 280 nm and 650 nm. The detected values can be used for DOL calculations, description stated in the according labeling manual.
6. Optional*: Fluorescence detection is performed with a Jasco FP-8300 fluorescence spectrometer (JASCO), including the flow cell in the flow after the column using versatile valves. To detect intrinsic protein fluorescence, select the emission intensity mode. The excitation wavelength is set to 280 nm and emission to 330 nm.

For most systems only one fluorescence signal can be detected at a time. An alternative approach is to detect NanoTemper RED dyes. For this, set the excitation wavelength to 620 nm and emission to 670 nm. The excitation bandwidth is set to 20 nm, emission – 5 nm for 330 nm and 20 nm for 670 nm. The response time is set to 1 second and detector sensitivity mode is 'High'. The signal - voltage on the detector was transmitted to the Unicorn software as an analog signal.

7. Fractions containing the labeled membrane protein are collected and pooled.
8. Concentration and DOL are determined.

D. Contributors

Anton Turaev[1], Hanna Kratzat[1]

[1] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com

* The optional fluorescence detection measurement in step 6 can be used if the protein concentration is lower than recommended in the protocol but may not be necessary.