LigandTracer

Protocol

Protein labeling with FITC

This protocol describes labeling of proteins with Fluorescein isothiocyanate (FITC), suitable for measurements with LigandTracer® Green. The protocol may also be used as a basis for labeling procedures with other amine-reactive dyes, such as ATTO- or Alexa Fluor NHS-ester dyes.

Important information

Sodium azide, carrier proteins and amino acids can interfere with the labeling procedure and should be removed prior to labeling, e.g., by buffer exchange against the labeling buffer. In such a case, start at step 2 in the protocol below.

Note that FITC is typically conjugated via primary amines (lysines) and may affect the binding properties of the protein.

Materials

- Protein (preferably at least 1 mg/ml in stock solution and a total quantity of 30-600 µg)
- FITC (light sensitive)
- Dimethyl sulfoxide (DMSO)
- Labeling Buffer: 0.5 M Sodium carbonate-bicarbonate buffer pH 9.5 or 50 mM Borate buffer pH 9
- Gel filtration column, Sephadex G-25 (such as NAP™-5) or similar
- Storage buffer, e.g., PBS

Procedure

1. Dilute protein in labeling buffer to 0.3- 2 mg/ml (down to 0.1 mg/ml is possible if stock concentration is low) with a total reaction volume of 300 µl.
   a. For sodium carbonate bicarbonate buffer: Dilute the protein directly in the labeling buffer. For pH-sensitive proteins, 0.1 M Sodium carbonate-bicarbonate buffer pH 9.2 can be used instead, as long as the labeling buffer constitutes of at least 50 % of the final volume of the protein/buffer solution.
   b. For borate buffer: Add one volume of protein solution to two volumes of borate buffer (dilute protein in PBS if needed).
2. Dissolve FITC in DMSO to a concentration of 1 µg/µl. The reactive FITC molecule is unstable and should be used immediately after it has been solubilized. Discard any excess FITC solution after labeling. Degree of labeling (DOL) can be optimized by using a higher or lower FITC to protein ratio. For antibodies, we typically aim for a DOL of 2-3.
3. Add the FITC solution to the protein/buffer solution to get a final concentration of 100 ng FITC/µg protein. Mix immediately.
4. Wrap the tube in foil and incubate at 37 °C for 90 min.
5. Remove excess FITC and exchange the protein into storage buffer (e.g., PBS) by gel filtration with a Sephadex G-25 column (such as NAP™-5) or similar.
6. Fractionate the protein in small aliquots and store at -20°C. The labeled protein is sensitive to light and repetitive freeze-thaw procedures. Siliconized or protein low-bind tubes may be used to reduce the risk of unspecific binding during storage.