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 fluorophores such as Texas Red® or Alexa Fluor® 488, with proper adjustments to an ideal labeling degree (system dependent).

Important information

Sodium azide can react with the fluorophore and prevent conjugation. If the protein solution contains azide, we recommend a buffer exchange directly against borate buffer pH 9. In such a case, start the protocol below from step 3.

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

Materials

- Protein (preferably at least 0.5 mg/ml in stock solution):
  -For antibodies or proteins of ~150 KDa: 20-100 µg
  -For other molecular weights: Aim at a final concentration of 100-600 nM in 1 ml labeled solution
- FITC (light sensitive)
- Dimethyl sulfoxid (DMSO)
- Borate buffer pH 9
- Gel filtration column, e.g. NAP™-5
- Storage buffer, e.g. PBS

Procedure

1. Dissolve protein to a concentration of 1-2 mg/ml in PBS (if protein stock has a higher concentration).
2. Add one volume of protein in PBS to two volumes of borate buffer.
3. 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.
4. Add the FITC solution to the protein/borate solution to get a final concentration of 100 ng FITC/1 µg protein. Mix immediately.
5. Wrap the tube in foil and incubate at 37°C for 90 min.
6. Remove excess FITC and exchange the protein into storage buffer (e.g. PBS) by gel filtration, e. g. with a NAP-5 column.
7. Fractionate the protein in small aliquots and store at -20°C. The labeled protein is sensitive to light and to repeated freeze-thaw procedures. Siliconized tubes may be used to reduce the risk of unspecific binding during storage.