

Labeling Protein with Thiol-reactive Probes

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[Abstract] This protocol is used to label protein or peptide with a maleinimide or iodoacetamide conjugated fluorescent probe through the free cysteine.

Materials and Reagents

1. Thiol-reactive Maleimide probes (Life Technologies, Invitrogen™)
Note: A large collection of probes are available from Invitrogen.
2. Tris-(2-carboxyethyl)phosphine (TCEP) (Life Technologies, Invitrogen™, catalog number: T2556)
3. Dithiothreitol (DTT) (Life Technologies, Molecular Probes®, catalog number: D1532)
4. DMSO
5. Dissolving buffer (see Recipes)

Equipment

1. Aluminum foil
2. Sephadex G-25 column

Procedure

1. Dissolve protein at 50-100 μ M in dissolving buffer containing 1 mM TCEP at room temperature to keep reactive cysteine reduced.
Note: It is not necessary to remove excess TCEP during conjugation with iodoacetamides or maleimides. TCEP can be substituted with DTT. If DTT is used, then dialysis is required to remove the excess DTT prior to introducing the reactive dye.
2. A 1-10 mM stock solution of thiol-reactive probe is prepared in DMSO immediately prior to use. Protect stock solution from light by wrapping containers in aluminum foil.
3. Mix thiol-reactive probe and protein as approximately 10:1 molar ratio and allow the reaction to proceed preferably in dark for 2 h at room temperature or overnight at 4 °C.
Note: Add the probe into protein solution dropwise and it is stirring.
4. Upon completion of the reaction, the conjugate is separated from excess dye on a gel filtration column (e.g. a Sephadex G-25 column) or by extensive dialysis at 4 °C in an appropriate buffer.

Recipes

1. Dissolving buffer (pH 7.0-7.5)
10-100 mM phosphate
Tris
HEPES

References

1. Hermanson, G., (1996). Bioconjugate Techniques, Academic Press.