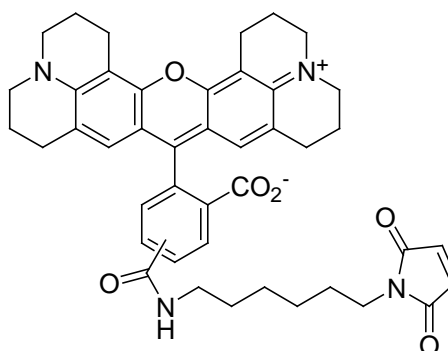


PRODUCT AND SAFETY DATA SHEET

PRODUCT NAME: 5(6)-ROX maleimide**CATALOG #** 91055**MOLECULAR INFORMATION:** C₄₄H₄₈N₄O₆
MWt: 712**PROPERTIES:****Color & Form**
SolubilityDeep purple solid
Soluble in DMSO, or DMF**STORAGE AND HANDLING:**

Store desiccated at -20 °C. Protect from light, especially in solution. When stored as a solid at -20 °C, the product is good for at least two years. When dissolved in anhydrous DMF or DMSO and stored at -20 °C, the product should be stable for at least a month.

APPLICATION:

5(6)-ROX maleimide is a long wavelength thiol-reactive rhodamine dye. The C6 spacer arm between the dye and the maleimide reactive group enhances the fluorescence of the dye when the dye is conjugated to proteins. Maleimides can selectively label thiols in the presence of amines when the pH is controlled around 7, where the amines are mostly protonated and thus unreactive.

The dye is a mixture of 5- and 6-isomers, which is suitable for protein labeling, or labeling of other macromolecules where either isomer dye produces the same result. For application where a single isomer is required, please contact Biotium for availability and price.

Recommended Procedure for Labeling Proteins:¹

- 1) Dissolve the protein at 50-100 µM concentration in a pH 7.0-7.5 buffer (for example, 10-100 mM phosphate, Tris, or HEPES).
- 2) If necessary, disulfide bonds can be reduced to thiols for labeling by using a reducing agent (10-times excess) such as TCEP.²
- 3) If DTT is used in step 2) and the excess DTT has been removed by dialysis, it may be necessary to carry out the labeling reaction under nitrogen or argon to prevent disulfide formation during the reaction. Thus, after the disulfide reduction reaction, the protein solution needs to be deoxygenated by passing nitrogen or argon through the solution for about 10 minutes and then the solution is kept under the inert gas. If TCEP is used in step 2, excess TCEP

can prevent disulfide formation and therefore an inert gas may not be necessary.

- 4) Prepare a 10-20 mM stock of the dye in DMF or DMSO.
- 5) Add the dye stock solution dropwise to the stirred protein solution. In general, the molar ratio of dye to protein should be around 10-20 to 1.
- 6) Continue to stir the resulting solution in the dark for 2 hours at room temperature. For proteins that are unstable at room temperature, the reaction may be carried out at 4 °C overnight.
- 7) To quench the reaction, an excess of a small thiol compound such as mercaptoethanol or glutathione is added to react with the excess dye.
- 8) Separate the product by gel filtration or dialysis.

NOTES: 1) procedures for labeling other biomolecules are similar. 2) If DTT is used as a reducing agent, it is necessary to remove the excess DTT by dialysis at the end of the reduction reaction because DTT can also react with maleimide. However, if TCEP is used, excess TCEP does not need to be removed for the subsequent step.

TOXICITY: Not established

FIRST AID:	Potentially harmful. Avoid prolonged or repeated exposure. Avoid getting in eyes, on skin, or on clothing. Wash thoroughly after handling. If eye or skin contact occurs, wash affected areas with plenty of water for 15 minutes and seek medical advice. In case of inhaling or swallowing, move individual to fresh air and seek medical advice immediately.
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