

Notes:

Keep peptide solutions as cold as possible. Freeze at -80C ASAP. Whenever possible, purge oxygen from solutions, centrifugation at max speed for a minute removes dissolved gas. To minimize O₂ blowing off with N₂ or Argon is a good thing.

Keep maleimide solutions as dry as possible. Do not open tubes when cold, to avoid condensation. Aliquot and freeze in prechilled dessicant at -20C. To thaw remove tube from frozen dessicant and place in room temp dessicant in the dark.

Keep fluorophore solutions in the dark whenever practical, dim lights when fluorophore is exposed

For each 100 uM reaction you will need:

20 nmoles fluorophore, 5 ul of a 4 mM stock

-make for fluorescein maleimide (Fmal) and tetramethyl rhodamine maleimide (TMRmal)

-dissolve in dry DMSO

5 nmoles (20 ug) GxTX peptide with spinster cysteine,

-20 ul of a 25 uM solution in 50% ACN + 1 mM EDTA pH 5 on ice.

25 ul 200 mM Tris, 20 mM EDTA pH 6.8

50 ul 20% ACN, 0.1% TFA

2 clear 1.5 ml tubes

ice bucket

a dark place

1.5 ml centrifuge, preferably chilled to 4°C

spectrophotometer, nanodrop is fine

Setup Controls as follows:

Tube 1: no GxTX no fluor (20 ul 50% ACN + 1 mM EDTA, 5 ul DMSO)

Tube 2: GxTX, no fluor

Tube 3: Fmal alone

Tube 4: TMRmal alone

Tube 5: GxTx + Fmal

Tube 6: GxTx + TMRmal

Protocol:

Place 20ul peptide in 1.5ul tube

Add 25 ul of 200 mM Tris, 20 mM EDTA pH 6.8

Add 5 ul of (1 mM solution of maleimide fluorophore in DMSO), pipet slowly until well mixed, avoid mixing air in.

Centrifuge 1 min max speed in centrifuge, note any precipitate.

React overnight at 4C.

Dilute with 50 uL 20% ACN, 0.1% TFA, mix well, spin-down, remove supernatant, spin again.

Quantify A280 and absorbance of fluorophore

Inject 50 ul supernatant onto HPLC, followed by 200uL of 20% ACN, and .1% TFA.

Quantify A280 and absorbance of fluorophore, record peptide concentration and degree of labeling.

Glossary

ACN = acetonitrile