

*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 O<sub>2</sub> blowing off with N<sub>2</sub> 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 ul reaction you will need:

100 nmoles fluorophore, 10 ul of a 10 mM stock

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

-dissolve in dry DMSO

20 nmoles (80 ug) GxTx peptide with spinster cysteine,

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

20 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: GxTx + Fmal

Tube 2: GxTx + TMRmal

Tube 3: no GxTx + Fmal (20 ul 50% ACN + 1 mM EDTA instead of GxTx)

Tube 4: no GxTx + TMRmal (20 ul 50% ACN + 1 mM EDTA instead of GxTx)

*Protocol:*

Place 20ul peptide in 1.5ul tube

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

Add 10 ul of (10 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 ~99 ul supernatant onto HPLC (save a tiny drop for mass spec, followed by 150uL of 20% ACN, and .1% TFA.

run HPLC protocol collect 1 ml fractions from tubes 1 and 2, take 5 ul sample of interesting fractions for mass spec and freeze remainder at -80C.

HPLC protocol:

time 0: 20% ACN

time 1: 20% ACN

time 2: 30% ACN

time 22: 35% ACN

time 24: 95% ACN

time 25: 95% ACN

time 26: 20% ACN

time 30: 20% ACN, end, ready for next run

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

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*Glossary*

ACN = acetonitrile