PEAS Labeling Gcn4-cys

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Notes

- 10 mCi 125I = 4.6 nmol (see spec. sheet for calculation)
- 4.6 nmol Gcn4(1-118,209-281) = 103 microgram (M.W. 22,400)
- PEAS has low solubility in aqueous solution
- Minimize the time Gcn4 is without DTT by preparing the protein just before labeling
- I have observed the best efficiency of labeling using a ratio of 1:4:1 (125I:PEAS:Gcn4); however, I would suggest a ratio of 1:1:1 next time
- The protocol below is from a labeling I did of six Gcn4 derivatives. The volumes of PEAS, 125I, sodium phosphate, and Tyrosine will have to be adjusted based on the number of proteins to be labeled and the ratio of 125I:PEAS:Gcn4 used. The ratio used for this labeling was 1:4:1.
- Phosphorimager cassettes do not shield 125I emissions

Buffers

Gcn4 dilution buffer: 20 mM HEPES, 0.15 M KCl, 1 mM EDTA, 10% glycerol, pH 7.9 (no DTT or PMSF)

0.1 M sodium phosphate pH 7.4

10 mM Tyrosine (store -20 deg)

Prepare Gcn4

1. Quantitate proteins to be labeled and determine the quantity of material to be used (recovery from Zebra gel filtration columns is about 75%)
2. If necessary, concentrate Gcn4 using Amicon Ultra-10 by standard protocol
3. Remove DTT from Gcn4 using Zebra 0.5 ml Desalt Spin Column equilibrated and eluted with Gcn4 dilution buffer
4. Transfer volume of Gcn4 to be labeled to a 1.5 ml tube, freeze remaining Gcn4 and store at -70 deg
5. Keep Gcn4 for labeling on ice

In the Hot Lab

1. Dilute PEAS 1:100 in DMSO (5.75 nmol/microliter final)

2. Add 0.1 M sodium phosphate to Iodogen tube followed by diluted PEAS so that the total volume added to the reaction is 57.7microliter, then mix gently
3. Add 10 mCi 125I (23 microliter, 4.6 nmol) to the Iodogen tube, mix gently
4. Incubate the reaction 4' at RT
5. Transfer 125I-PEAS reaction to a 1.5 ml Eppendorf tube containing 9.3 microliter 10 mM Tyrosine (use a ratio of 5:1 Tyrosine:PEAS)
6. Transfer desired amount of 125I-PEAS to Gcn4 samples, mix gently
7. Incubate reactions 30' at RT
8. Stop reactions and remove unincorporated PEAS by running reactions through a Zebra 0.5 ml Desalt Column equilibrated and eluted with Gcn4 dilution buffer
9. Collect elution in 1.5 ml tube and aliquot as desired
10. Assay incorporation using Hot Lab instrument: measure the activity of an aliquot of known volume
11. Freeze labeled proteins and store in lead box kept at -70 deg

Determining Concentration

Determine concentration and observe PEAS label by SDS-PAGE, CBB staining, and MR film

Run labeled proteins +/- DTT along with a titration of Gcn4 on a 4-12% Bis-Tris gel in MES buffer (see example)

Running buffer will be hot- collect and dispose as radioactive waste

Scan CBB stained gel on Odyssey in 700 channel and quantitate

Dry gel and expose to MR film for 20' or less