FECH Activity Assay

FERROCHELATASE (FECH)

Sample preparation

1. Suspend ~50-µL mammalian cell pellet in 400µL TGD buffer (Tris buffered glycerol with dithiothreitol (DTT), made by dissolving 2mL or 2.52 g glycerol and 1.5mg DTT in 8.0 mL 20mM Tris pH 8.0)
2. Sonicate (homogenize) while in an ice bath at the lowest practicable power setting for 3 cycles x 5 sec at 50% duty (2.5 sec on, 2.5 sec off)
3. Determine the protein content and dilute to 1µg protein/µL with more TGD

Ferrochelatase reaction

1. Prepare three 50-µL aliquots of the cell preparation, two live and one inactivated in boiling water for 10 minutes (as control for non-enzymatic product formation).
2. Prepare the incubation buffer containing 160mM Tris pH 8.0, 40mM Bicine pH 8.0, 10mg/ml Tween20 and 0.38mg/mL palmitic acid),
3. Mix 150µL incubation buffer and 25µL zinc substrate (1mM aqueous Zn acetate).
4. Mix each 50-µL aliquot of cell preparation with the 150µL incubation buffer plus zinc substrate and pre-incubate for 5 minutes at 37˚C.
5. Then add 25 µL of mesoporphyrin IX substrate (250µM in 160mM Tris pH 8.0, 40mM Bicine pH8.0, 2mg/ml Tween20).
6. Incubate the mixture for 30 min at 37˚C.
7. Add 750µL stop reagent (270µM ethylenediaminetetraacetic acid in a mixture containing dimethylsulfoxide-methanol, 30/70 by volume respectively).
8. Cool on ice for 15-20 min.
9. Centrifuged at 1500xg for 10 min at room temperature.

Quantitation of product Zn mesoporphyrin IX

1. Inject 10µL of supernatant solution of porphyrins into a Waters Acquity UPLC (ultra performance liquid chromatography) system, which includes a binary solvent manager, sample manager, fluorescence detector (FLR), column heater and an Acquity UPLC BEH C18, 1.7 µM, 2.1 x 100 mm column.
2. Set the FLR for zinc mesoporphyrin IX (ZnMeso) at 406 nm excitation and 578 nm emission.
3. Quantify the ferrochelatase product relative to a standard ZnMeso solution, also in the stop reagent.

4. Keep the sample chamber dark and at ambient temperature.
5. Solvent A is 0.2% aqueous formic acid while Solvent B was 0.2% formic acid in methanol.
6. Set the flow rate at 0.40 mL per minute at 60˚C for the total run time of 7 min.
7. Use the following successive gradient settings for run time in minutes versus A:
    - 0.0, 80%
    - 2.5, 1%
    - 4.5, 1%
    - 5.0, 80%
8. Keep all solvent gradients are linear.

Revision #2
Created 1 December 2023 21:55:16 by Elliot Francis
Updated 4 December 2023 16:26:14 by Elliot Francis