

# FECH Activity Assay

## FERROCHELATASE (FECH)

### Sample preparation

1. Suspend ~50- $\mu$ L mammalian cell pellet in 400 $\mu$ L 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 1 $\mu$ g protein/ $\mu$ L with more TGD

### Ferrochelatase reaction

1. Prepare three 50- $\mu$ L 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 150 $\mu$ L incubation buffer and 25 $\mu$ L zinc substrate (1mM aqueous Zn acetate).
4. Mix each 50- $\mu$ L aliquot of cell preparation with the 150 $\mu$ L incubation buffer plus zinc substrate and pre-incubate for 5 minutes at 37°C.
5. Then add 25  $\mu$ L of mesoporphyrin IX substrate (250 $\mu$ M 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 750 $\mu$ L stop reagent (270 $\mu$ M 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 10 $\mu$ L 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  $\mu$ M, 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.
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