

Reverse FECH

- Sonicate the washed pellet of cultured bacterial cells in two volumes of 10mM KPi pH 5.5. Determine protein content and adjust to about 20mg/mL with the same buffer to prepare the homogenate. Deoxygenate all samples and reagents.
- Mix about 50 μ L homogenate (1.0 mg total protein) with 50 μ L of assay reagent containing 100 μ M hemin-imidazole, 4 mM ascorbic acid in 10 mM potassium phosphate buffer pH 5.5 under argon atmosphere.
- Cap the sample tubes securely, incubate for 1h at 45°C in a water bath and then add 400 μ L 50% v/v acetone in ethanol.
- Centrifuge the samples at 13,500 g for 10 min.
- Analyze the supernatant for PPIX fluorescence by UPLC (ultra-performance liquid chromatography).
- For blank controls samples of bacterial homogenates were heated for 10 min in a boiling water bath and assayed with the live samples

Revision #2

Created 2023-12-01 21:53:59 UTC by Elliot Francis

Updated 2023-12-04 16:09:20 UTC by Elliot Francis