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 50µL homogenate (1.0 mg total protein) with 50 µL of assay reagent containing 100 µM 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 µL 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

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