Protocol for reverse FECH assay. Hector Bergonia 03-26-20

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 in pairs.