

PORPHOBILINOGEN DEAMINASE (PBGD)

PBGD Assay

Add 10 μ L homogenized sample to 75 μ L 10 μ M dithiothreitol in 0.1M Tris pH 7.65.

Perform subsequent steps in the dark until addition of HCl.

Add 15 μ L 2.2mM PBG (porphobilinogen) in 0.1M Tris pH7.65.

Incubate in a 37 $^{\circ}$ C water bath for 30 min.

Stop the reaction with 100 μ L 3M HCl.

Expose the mixture to UV light for 30 min or ambient light for 2h to oxidize all porphyrinogen formed.

Centrifuge at 16000xg for 10 min. Collect the supernatant.

Quantify the resulting porphyrins by UPLC (ultra performance liquid chromatography).

For blanks use samples inactivated in boiling water for 10 min or live samples at 0 min incubation with PBG.