

PBGD Activity Assay

PORPHOBILINOGEN DEAMINASE (PBGD)

PBGD Assay

1. Add 10 μ L homogenized sample to 75 μ L in 0.1M Tris pH 7.65.
2. Add 15 μ L 2.2mM PBG (porphobilinogen) in 0.1M Tris pH7.65.
3. Incubate in a 37°C water bath for 30 min.
4. Stop the reaction with 100 μ L 3M HCl.
5. Expose the mixture to UV light for 30 min or ambient light for 2h to oxidize all porphyrinogen formed.
6. Centrifuge at 16000xg for 10 min. Collect the supernatant.
7. 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.

For purified PBGD preparations, use 10 μ L 0.05mg/mL instead of the 10 μ L homogenized sample.

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