

Guanylyl Cyclase Assay

- Use Tris-ICB as buffer, at desired calcium level.
- Use alpha-toxin at 100 U/ml, usually 250 μ l /well in Tris-ICB with 0.1% BSA and 25 mM glucose.
- Let incubate at 37°C for about 30 minutes.
- Test with Trypan Blue.

- Use 100 μ l /well of reaction mix - Same as adenylate cyclase except use cGMP instead of camp
- Quench with 250 μ l 200 mM Zn Acetate
 - Cannot use GTP to quench as it precipitates out of solution
- Use 1% SDS and 1 mM cGMP
- Add 200 mM (250 μ l) Na Carbonate
- Freeze at -70C.

- Scrape and rinse with 500 μ l ddH₂O into eppendorfs.
- Spin and transfer supernatant into 12 x 75 mm glass tubes.
- Add 1 ml perchloric acid.
- Apply to fresh, unwashed 0.5-0.6g alumina columns.
- Wash with 10 ml ddH₂O.
- Elute with 3 ml of 0.2 M Ammonium Formate.