

## **Anthrone Reaction for Polysaccharide Detection**

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The anthrone reaction can be used to quantify the amount of polysaccharide in a test solution, although the sensitivity of this assay is fairly low

### **Materials**

- Assay “plates”—we prefer to use the 1.1 ml mini-tubes and racks from VWR (Catalog# 89092-228). You need something that can hold up to 150 microliters of volume and can be safely put into a boiling water bath.
- ELISA plates (any)
- ELISA plate reader with 630 nm filter (any)
- Water bath (any, set at 100°C)
- Pipettors and pipet tips (any, various sizes)
- Concentrated sulfuric acid (any)
- Anthrone (we use Sigma A1631)

### **Reagents**

#### Anthrone reagent

Add 0.2 grams of anthrone to 100 ml concentrated sulfuric acid. Can be stored at room temperature for 2-3 days.

#### Standard

Known concentrations of PS (0, 50, 100, and 200 mg/L) in water can be used as a standard.

### **Procedure**

1. Prepare 100°C water bath.
2. Add 50 microliters of the polysaccharide-containing solution (test sample, standard, or water for a blank) into plastic mini-tubes.
3. Add 100 microliters of anthrone reagent into each mini-tube. Use caution—tubes will get hot.
4. Mix the reagents well by pipetting them up and down CAREFULLY.
5. Place tubes, uncovered, into a 100°C water bath for 5 min.
6. Remove the tubes and let them cool for ~5 minutes at room temperature.
7. Transfer 100 microliters from each well into the corresponding well of an ELISA plate.
8. Read the OD at 630 nm.
9. By comparing the ODs of an unknown to those of a standard, the polysaccharide concentration can be determined.

Note: Strong acid is used. Wear eye protection and other personal protective equipment. Also, dispose of the reaction products carefully. Assay sensitivity is about 50-100 mg/L of capsule PS.