E. coli Optical Quantification

Version 2

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481b Laboratory

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ABSTRACT

This procedure details how to culture *E. coli* K-12 in suspension and quantify cultured bacteria using a spectrometer.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

Labcoat and gloves must be worn at all times.

MATERIALS TEXT

- Gloves
- Lyophilized *E. coli* K-12
- BHI Broth
- 15 mL centrifuge tubes
- Pipette and tips
- 70% Ethanol solution
- Kimwipes®
- Mini Spectrometer
- Cuvettes
- Computer with OceanView software

Inoculate Culture

1 Pipette **10 ml** BHI broth into 15 mL centrifuge tube.

2 Place tube in incubator or water bath to warm.

3 Add **2 mg** lyophilized *E. coli* K-12 to warm BHI broth.

4 Close lid and shake gently.

5 Incubate at **37 °C** for several hours.

Quantify Concentration

6 Dispense 1 ml BHI broth into cuvette.

> NOTE
> This should be broth *without* bacteria and will serve as your reference for calculating absorbance.

7 Carefully insert cuvette into spectrometer.

> NOTE
> Wipe cuvette with delicate task wipes if necessary.

8 Measure intensity at 600 nm.

9 Repeat Steps 6-8 with the samples cultured for different durations.

> NOTE
> Avoid pipetting any precipitation at the bottom of the culture tube. Draw only supernatant.

10 Using the BHI broth without bacteria as I_0, calculate the absorbance of each of the samples.

11 Plot Absorbance as a function of time cultured. **Include this plot, and the table of intensities and calculated absorbances in the 'Results' section of your lab report.**