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E. coli Optical Quantification V.2

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We use this protocol and it's working

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Abstract

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

Guidelines

Labcoat and gloves must be worn at all times.

Materials

- 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.**