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Version 1

E. Coli Optical Quantification V.1

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Protocol status: Working

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.**