Institut für HIV Forschung

SOP #05-00 (Februar 2016)

DNA/RNA measurement with Biospectrophotometer

Wear Gloves.

- 1. Turn on the Biospectrophotometer using the switch on the back
- 2. Choose your settings, the Biospectrophotometer can measure several different pathlengths (such as 1cm cuvettes) but typically measurements are performed with the μ Cuvette (1mm path length)
- 3. Blank the machine
 - a. Clean the μ Cuvette with distilled water and a tissue
 - b. Load 2µl of buffer that sample is diluted with onto the small circle
 - c. Close the μ Cuvette and verify that liquid is making contact with both sides of the μ Cuvette
 - d. Load the μ Cuvette into the reader slot, close the lid, and press "Blank"
 - e. Re-blank after every 10 samples measured
- 4. Optional Verify quality of Blank
 - a. Clean the μ Cuvette with distilled water and a tissue
 - b. Load 2µl of buffer that sample is diluted with onto the small circle
 - c. Close the μ Cuvette and verify that liquid is making contact with both sides of the μ Cuvette
 - d. Load the μ Cuvette into the reader slot, close the lid, and press "Measure"
 - e. If you do not receive a value very close to 0 (within 0.5), then vortex your buffer and blank again
- 5. Vortex or pipette mix your DNA/RNA samples
- 6. Measure your sample concentration and purity
 - a. Clean the μ Cuvette with distilled water and a tissue
 - b. Load $2\mu l$ of sample is onto the small circle
 - c. Close the μ Cuvette and verify that liquid is making contact with both sides of the μ Cuvette
 - d. Load the μ Cuvette into the reader slot, close the lid, and press "Sample"
 - 260/280 window: a value of ~1.8 indicates pure DNA and a value of ~2.0 indicates pure RNA. Lower values indicate contaminants such as protein.
 - 260/230 window: a value of 1.8-2.2 indicates pure DNA/RNA. Lower values indicate contaminants
 - e. Values are recorded on the machine and can be transferred to a USB, but due to the difficulty of typing in your sample names, writing down your samples/concentrations is highly recommended
- After measuring all of your samples, clean the μCuvette with distilled water and a tissue. Return it to its box and place the box on the shelf above the Biospectrophotometer
- 8. Turn off the Biospectrophotometer and clean the area

^{*}If decontamination is necessary, use a 5.25% solution of sodium hypochloride (bleach freshly prepared).