NanoDrop Measurement

This protocol describes the measurement of nucleic acid concentration and purity with a NanoDrop spectrophotometer.

Materials

- Sample to be measured
- Blank: Milli-Q water or elution buffer
- Lint-free tissues

Equipment

- NanoDrop spectrophotometer and computer
- Pipette and pipette tips (0.5–10 µL)

Use the same buffer for the blank as the sample is dissolved in.

Protocol

1. Start the NanoDrop software on the computer.
2. Raise the arm and clean the measurement pedestal with a lint-free tissue.
3. Pipette 1 µL of the blank onto the pedestal and lower the arm.
4. Press *Blank*.
5. Clean the pedestal again and pipette 1 µL of your sample.
6. Press *Measure* and note the concentration in ng/µL together with the 260/280 and 260/230 ratios.
7. Clean the pedestal and the arm when you are finished.

For pure DNA the 260/280 ratio is around 1.8 (around 2.0 for RNA), and the 260/230 ratio is 2.0–2.2. Lower values indicate contamination with protein, phenol or salts

OD600 Measurement

This protocol describes the measurement of culture density at 600 nm with a cuvette spectrophotometer.

Materials

- Culture to be measured
- Sterile medium (same as culture is grown in)

Equipment

- Spectrophotometer
- Cuvettes
- Pipette and pipette tips (100–1000 µL)

Protocol

1. Add 1 mL of sterile medium to a cuvette.
2. Insert the cuvette into the spectrophotometer and press *Blank*.
3. Add 1 mL of culture to a second cuvette.
4. Insert that cuvette and press *Sample*.
5. Note the absorbance at 600 nm.

Most spectrophotometers are only linear up to an OD_{600} of about 1. Above that, dilute the culture in medium, measure again, and multiply the reading by the dilution factor

Qubit Measurement

This protocol describes the fluorometric quantification of DNA, RNA or protein with a Qubit fluorometer, which is more accurate than absorbance for low concentrations.

Materials

- Sample to be measured
- Qubit assay kit for the analyte and range (e.g. dsDNA HS or BR): reagent, buffer and two standards

Equipment

- Qubit fluorometer
- Qubit assay tubes (0.5 mL, thin-walled)
- Vortex
- Pipettes and pipette tips (0.5–10, 20–200 µL)

Bring the buffer, reagent and standards to room temperature before starting, and protect the reagent from light.

Protocol

1. Prepare the working solution by diluting the Qubit reagent 1:200 in Qubit buffer. You need 200 µL per tube, so prepare enough for all samples plus the two standards.
2. Label the assay tubes on the lid, not the side, so the fluorometer can read through the tube wall.
3. Standards: add 190 µL working solution and 10 µL of standard 1 to the first tube, and 190 µL working solution and 10 µL of standard 2 to the second.
4. Samples: add 1–20 µL of sample to each tube and top up with working solution to 200 µL.
5. Vortex all tubes for 2–3 s and avoid bubbles.
6. Incubate for 2 min at room temperature, protected from light.
7. On the fluorometer, select the assay used, run the two standards first, then read each sample.
8. Enter the volume of sample added so the instrument reports the stock concentration, and note the result.

Read the tubes within the time stated for the assay, since the signal drifts afterwards. Run fresh standards each time a new kit is opened