



Quantification of nucleic acid, RNA and oligonucleotides

1. Subject

In some experiments it is necessary to know the exact amount of ribosomal DNA. There are all kinds of methods to measure DNA quantity. In this protocol a UV spectrophotometer is used. The advantage is that no coloration of fluorescent label is used.

2. Principle

ssDNA, dsDNA, RNA, proteins and other carbohydrates absorb light in the range of 230 to 280 nm. The quantity of DNA can be measured at 260 nm. An OD₂₆₀ of 1 means a dsDNA concentration of 50 ng/ μ l and 33 ng/ μ l of ss DNA. The purity of the sample is measurable by the ratio of OD₂₆₀/OD₂₈₀. Proteins absorb at 280 nm. The OD₂₆₀/OD₂₃₀ ratio shows the contamination of carbohydrates, phenols, peptides, aromatic compounds.

3. Reagents and material

- 3.1 Tris(hydroxymethyl)-aminomethane (Merck 8382)
- 3.2 1N HCl (Ferak 11448)
- 3.3 spectrophotometer (Perkin-Elmer, Lambda 15 UV/VIS Spectrophotometer)
- 3.4 pipets and tips
- 3.5 two quartz cuvetts 600 μ l

4. Solutions

- 4.1 Tris buffer 10 mM, pH 8:
Add 0.12 g Tris [3.1] to 80 ml ultrapure water. Adjust pH to 8.0 with 1N HCl [3.2]. Adjust volume to 100 ml with ultrapure water. Aliquot the buffer in 50 ml tubes. Autoclave the solution during 15 min. at 121°C. Store at roomtemperature.

5. Protocol

- 5.1 Switch on the spectrometer; warming up takes about 3 min..
- 5.2 Date and time can be inserted, but is not necessary to operate the spectrometer.
- 5.3 Key: 4-line, Page, 6-line, off (the recorder is switched off), 8-line.
230, ABSC 1 to program first wave length
260, ABSC 2 to program second wave length
280, ABSC 3 to program third wave length
(300, ABSC 4 to program fourth wave length); this is optional.
- 5.4 Fill 2 quartscuvetts with 600 μ l Trisbuffer 10 mM, pH 8 [4.1] and place into the spectrometer.
- 5.5 Press RUN/STOP (green button) and when reaching 230 nm, press again.
- 5.6 Zero-adjust the spectrometer by pressing BACK/CORR (yellow button) twice.
- 5.7 Page, press RUN/STOP: this gives the blank values for each wavelength.
- 5.8 Pipet 5 μ l DNA-extract in the front cuvet and mix by tilting 3-4 times. Use parafilm to close cuvet. Replace cuvet into the spectrometer.
- 5.9 Press RUN/STOP: write down the 4 values for each wave length.
- 5.10 Repeat 5.8 and 5.9 for every next sample using the same cuvet without cleaning (samples are pooled).

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