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Quantitative Estimation of DNA

Practical 3 - Nucleic-acid measurement practical

Aim

To estimate nucleic-acid concentration and assess spectral quality.

Principle

At a 1 cm path length, an absorbance of 1 at 260 nm conventionally corresponds to about 50 micrograms/mL double-stranded DNA or 40 micrograms/mL RNA. These factors depend on nucleic-acid type; absorbance ratios alone do not prove purity or integrity.

Materials and Requirements

- Approved nonpathogenic teaching system or supplied anonymised dataset
- Laboratory risk assessment, controls and raw-data worksheet
- Approved method or teaching-data pack and a dated record sheet

Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Record nucleic-acid type, path length or instrument normalisation and the matching solvent blank.
3. Measure the full relevant spectrum and dilution within the qualified instrument range.
4. Calculate concentration with the correct factor and dilution; inspect 260/280 and 260/230 ratios with pH and blank context.
5. Use a gel or other orthogonal integrity and specificity check when required.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

Preparation / control	Condition / method	Raw observation	Calculated / interpreted result
Negative / vehicle control	Specify	Record	Evaluate
Positive / reference control	Specify	Record	Evaluate
Test replicates	Specify	Record	Calculate

Calculations

- Concentration = A260 x nucleic-acid factor x dilution factor, for absorbance normalised to 1 cm
- Yield = concentration x recovered volume

Worked Example

Illustrative double-stranded DNA: A260 = 0.20 and dilution 10 give 100 micrograms/mL. In 0.50 mL the yield is 50 micrograms.

Using the RNA factor would instead give 80 micrograms/mL. A DNA 260/280 ratio near 1.8 or RNA ratio near 2.0 is only a contextual indicator.

Result and Interpretation

- State nucleic-acid type, concentration, yield, spectral quality and independent integrity evidence.
- Report biological and technical replication separately and explain control performance.

Precautions

- Use only the approved biological system, containment and decontamination procedure.
- A teaching dataset does not authorise live animal, human-tissue or unknown-organism work.
- Keep original images and observations; avoid selecting only favourable fields or bands.

Viva Questions and Answers

Why use the correct factor?

Different nucleic-acid forms have different absorbance relationships.

Does a good ratio prove intact DNA?

Integrity needs another suitable measurement.

Why match the blank?

Buffer and pH can influence spectral measurements.

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