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Isolation and Estimation of RNA

Practical 21 - Approved nucleic-acid isolation practical

Aim

To isolate RNA from approved teaching yeast and assess concentration and integrity.

Principle

RNA purification requires effective nuclease control and separation from DNA, proteins and other matrix components. The extraction method and downstream use determine whether a DNA-removal step is required.

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. Use approved teaching yeast or supplied extraction records and document starting material.
2. Prepare a nuclease-controlled workspace and apply the adopted yeast-disruption and RNA-purification kit method.
3. Include extraction blanks and apply the justified DNA-removal and cleanup steps.
4. Measure concentration using an RNA-appropriate method and assess integrity by the selected electrophoretic or instrument method.
5. Record yield, purity, integrity and suitability for the planned downstream application.

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

- RNA concentration = A260 x 40 micrograms/mL x dilution, for 1 cm-normalised absorbance
- RNA yield = concentration x recovered volume

Worked Example

Illustrative A260 = 0.25 at dilution 5 gives 50 micrograms/mL RNA. A 0.20 mL recovery contains 10 micrograms.

Absorbance alone cannot distinguish residual DNA from the intended RNA.

Result and Interpretation

- Present extraction flow, recovery, purity indicators and gel evidence.
- Report biological and technical replication separately and explain control performance.

Precautions

- Use the approved RNase-control practices and compatible nuclease-free materials.
- Do not treat an acceptable absorbance ratio as proof of intact RNA.
- Retain the RNA-specific quality and DNA-contamination controls.

Viva Questions and Answers

Why control RNases?

RNA can degrade rapidly through nuclease activity.

Why assess DNA contamination?

It can bias concentration and downstream amplification.

What confirms RNA integrity?

A suitable electrophoretic or equivalent integrity method.

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