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Cell Subculture Records and Cytotoxicity Analysis

Practical 12 - Biological teaching-data practical

Aim

To compare cell-viability measurements and retain valid subculture records.

Principle

Trypan-blue exclusion assesses membrane integrity, MTT reflects a metabolic reduction response and SRB measures a protein-associated signal after the selected procedure. These endpoints are related but not interchangeable.

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. Use an approved cell line with identity and contamination-control records or a supplied dataset; review passage and subculture history.
3. Record treatment, vehicle, cell-free interference controls and assay-specific positive controls.
4. For dye exclusion count viable and nonviable cells using the defined chamber and dilution; for MTT/SRB verify blank correction and linear response.
5. Calculate each endpoint separately and compare independent biological replicates, recognising compound colour or chemical-reduction interference.
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

- Dye-exclusion viability (%) = viable cells / total cells x 100
- Relative corrected assay response (%) = (test - relevant blank)/(vehicle - relevant blank) x 100

Worked Example

Illustrative 180 viable and 20 nonviable cells give 90% viability. MTT corrected signals 0.40 test and 0.80 vehicle give 50% relative metabolic response.

Those values need not agree because they measure different attributes; an IC50 requires a justified concentration-response fit.

Result and Interpretation

• Report the endpoint, controls, replicates and interpretation without equating metabolic response directly to cell number.
• 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

What does trypan blue assess?

Loss of membrane exclusion under the defined timing.

Why use cell-free compound controls?

The compound may alter colour or assay chemistry.

Is an MTT reduction direct proof of apoptosis?

A mechanism-specific assessment is needed.

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