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Biomolecule Separation and Gel Densitometry

Practical 7 - Protein electrophoresis practical

Aim

To assess protein separation by SDS-PAGE and qualified gel densitometry.

Principle

SDS denaturation gives many proteins a broadly comparable charge-to-mass relationship, so mobility mainly reflects apparent molecular size. Reduction, glycosylation and unusual composition can change migration.

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 approved protein preparations with a molecular-size marker and stated reducing or nonreducing conditions.
3. Follow the selected gel, buffer, loading and electrical SOP and retain the complete lane map.
4. Stain and image within the unsaturated response range; record background subtraction and a suitable densitometric calibration.
5. Estimate apparent size from the appropriate marker region and quantify only within a validated linear signal range.
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

- Relative mobility = band distance / dye-front distance
- Fit log10 apparent molecular mass against relative mobility within the supported marker range.

Worked Example

Illustrative fit log10(kDa) = 2.0 - 1.0 x Rf. A band at Rf 0.50 gives about 31.6 kDa.

A single band does not establish molecular identity or absolute purity, and staining responses differ between proteins.

Result and Interpretation

- Report apparent size, lane evidence, densitometry controls and the limits of purity claims.
- 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 add SDS?

It denatures proteins and influences their charge-to-mass relationship.

What does reducing agent change?

It can break disulphide links under the adopted method.

Is one gel band definitive identity?

An independent identity method is needed.

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