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Western Blotting

Practical 19 - Biological teaching-data practical

Aim

To quantify a target protein from a controlled Western-blot dataset.

Principle

Western blotting combines separation, transfer and antibody-based detection. Specificity and a nonsaturated linear response are needed; normalisation corrects selected loading effects but cannot repair poor specificity.

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 records with target and loading or total-protein controls.
3. Assess full-lane separation, transfer controls, antibody specificity and the absence of saturated exposure.
4. Apply consistent regions and background subtraction and normalise with a justified stable loading measure.
5. Compare independent biological replicates and retain uncropped images and processing settings.
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

- Normalised target = background-corrected target signal / justified loading signal
- Relative fold = normalised test / normalised reference

Worked Example

Illustrative target/loading signals are 200/100 in control and 300/100 in test: normalised values 2 and 3 give a 1.5-fold difference.

Do not interpret a saturated band or unstable loading control quantitatively.

Result and Interpretation

- Report target specificity, linear range, normalisation and biological variability.
- 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 keep uncropped images?

They preserve lane context and additional signals.

What invalidates densitometry?

Saturation or an unvalidated response range can distort ratios.

Why distinguish biological replicates?

They represent independent biological observations rather than repeated readings.

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