ELISA Data Analysis Checklist

A one page reference for consistent results across plates, dates, and operators.

Before you analyze

☐ Confirm assay type (qualitative, semi quantitative, quantitative) and format (sandwich or competitive).

☐ Confirm read settings (primary wavelength, reference wavelength if used) and the timing window in the protocol.

☐ Confirm standards are prepared correctly (dilution series, units, matrix, replicate wells).

☐ Export the raw plate file and save an untouched copy with date, plate ID, and operator initials.

Raw data and background

☐ Review the plate map and confirm sample IDs match well positions.

☐ If a reference wavelength is specified, apply it consistently (for example 450 minus 570).

☐ Identify background wells (blank or zero standard) and subtract the background value from all wells.

☐ Scan for obvious artifacts (bubbles, fingerprints, splashed wells, reading errors) and flag affected wells.

Standard curve fit

☐ Choose an appropriate curve model for your assay (4PL is common, 5PL if clearly asymmetric).

☐ Verify standards are monotonic and bracket the expected sample range.

☐ Check standard replicates and remove a point only with a documented reason (not to force a better curve).

☐ Review fit quality using residuals and back calculated standards based on your lab acceptance criteria.

Dilution and sample handling factors

☐ Apply dilution factors after interpolation (example 1 to 10 dilution means multiply by 10).

☐ Document any additional processing factors (concentration steps, volume changes, unit conversions).

☐ If matrix effects are suspected, confirm that sample diluent and standards match the intended matrix as closely as possible.

Replicate quality checks

☐ Calculate mean, standard deviation, and coefficient of variation (CV) for samples and standards.

☐ Set a CV target that matches the study purpose. Many research workflows start at 20 percent or lower, tighter for high confidence work.

☐ If duplicates disagree, investigate before averaging (pipetting, wash performance, well artifacts, edge effects).

Controls and plate effects

☐ Verify negative controls remain near background and positive controls fall within the expected range.

☐ Check for row or column trends and edge effects, especially on outer wells.

☐ Confirm incubation conditions were stable (time, temperature, plate seal) and washing was consistent.

☐ If running multiple plates, include an inter plate control sample when possible.

Interpolate unknowns

☐ Interpolate concentrations only within the quantifiable range of the standard curve.

☐ Flag samples above range and plan a rerun at a higher dilution.

☐ Flag samples near background and treat them as below quantifiable range unless your protocol defines a valid approach.

☐ Report units clearly and keep them consistent across plates and studies.

Normalization, statistics, and reporting

☐ Normalize only when it supports the study design (for example inter plate control normalization across runs).

☐ Choose statistical tests that match the design (paired vs unpaired, number of groups, distribution assumptions).

☐ Record critical metadata with the results (kit lot, plate reader model, analysis software, curve model, analyst).

☐ Keep a clear audit trail from raw ODs to final concentrations.

Notes

Use with your kit protocol. Acceptance criteria can vary by assay format and intended use.
Aviva Systems Biology. Technical Resources: avivasysbio.com/technical-resources/elisa