How to Run an ELISA Assay: Invitrogen ELISA Kit Reference Guide

This guide explains how to run an ELISA assay using an Invitrogen ELISA kit, from unpacking the kit through plate reading and result interpretation. It follows the sandwich ELISA workflow demonstrated with the kit's pre-coated plate, standards, buffers, and detection reagents, and is organized in a validation-report format so each stage of the procedure and its expected outcome can be reviewed at a glance.

Objective

Assay name: Invitrogen sandwich ELISA kit (pre-coated capture antibody plate).

Intended use: Quantification of a target protein in prepared samples by comparison against a standard curve generated from kit-supplied protein standards.

Matrix: Samples, controls, and background wells prepared and diluted in kit-supplied Sample Diluent and Assay Buffer, alongside reconstituted protein standards used to build the calibration curve.

Validation goal: This document describes the operational steps for running the assay as demonstrated with the kit. It does not present a formal statistical validation study; a validation goal for this assay would typically define acceptance thresholds for accuracy, precision, specificity, linearity, range, robustness, and stability prior to routine use, which are addressed qualitatively in the Validation Design section below.

Method summary

Analytical principle: The assay uses a sandwich ELISA format. The pre-coated capture antibody on the plate binds the target antigen; a biotin-conjugated detector antibody then binds the antigen at a separate epitope, forming a "sandwich" complex. Streptavidin-HRP binds the biotin label, and a chromogenic substrate reacts with the HRP enzyme to generate a color signal proportional to the amount of antigen present.

Instrument platform: An ELISA plate reader is used to measure absorbance at 450 nanometers (A450). Automated plate washers may optionally be used during the wash steps; manual washing is also described.

Sample preparation: Protein standards are reconstituted and serially diluted (1:2 series, e.g., Std1–Std7 plus a background well). Samples and controls may require pre-dilution as specified in the kit protocol before being added to the plate.

Readout: Absorbance at A450 is recorded for each well and plotted against standard concentration (pg/mL) to generate a linear standard curve, which is then used to calculate protein concentration in unknown samples.

Validation design

The source procedure demonstrates assay execution rather than a formal validation study. The table below maps the procedural controls shown to standard validation parameters.

Parameter	Procedural control demonstrated
Linearity / range	1:2 serial dilution of standards (Std1–Std7) plus a background well establishes the calibration range plotted as absorbance vs. concentration.
Specificity	Sandwich format: capture antibody and biotin-conjugated detector antibody bind the antigen at distinct sites, reducing nonspecific signal.
Precision	Not addressed in this procedure. A precision study would require running replicate samples across multiple wells, plates, or lots and calculating %CV against a predefined acceptance limit.
Accuracy	Not addressed in this procedure. An accuracy assessment would compare measured concentrations of spiked or reference samples to known values.
Robustness	Kit-specific timing, temperature, and dilution instructions (e.g., 10-minute standard reconstitution, 30-minute substrate incubation in the dark) define the tested operating conditions;

	deviations from these were not evaluated.
Stability	Unused 8-well strips are resealed in the foil pouch and stored at 2–8°C for future use, indicating a reagent-stability handling requirement rather than a formal stability study.

Results

Stage 1: Kit contents and initial preparation

Step	Action	Observation
1	Review the kit-specific protocol before starting.	Assay-specific instructions confirmed prior to setup.
2	Unpack the kit and verify all components (pre-coated plate, standards, buffers, reagents).	All items present and undamaged.
3	Set up a clean workspace with a computer, pipettes, deionized water, and lab equipment.	Workspace ready for assay preparation.
4	Identify buffers and reagents (Wash Buffer 20X, Assay Buffer 20X, Sample Diluent, Substrate Solution) and check lot numbers and expiration dates.	Reagents confirmed valid for use.
5	Open the foil pouch, remove the plate, and take out unused 8-well strips; reseal and store unused strips at 2–8°C.	Plate ready with only the required strips in place.

Kit components, including the ELISA plate, labeled buffer bottles, standards, and reagents, arranged on the Invitrogen kit box with the on-screen title "Using your Invitrogen ELISA kit."

Laboratory workspace with benches, ELISA readers, a laptop displaying a plate layout, and a technician entering with the kit.

Close-up view of the ELISA plate and labeled buffer bottles: Wash Buffer 20X, Assay Buffer 20X, Sample Diluent, and Substrate Solution.

Gloved hands removing 8-well strips from the ELISA plate, with the kit box and foil pouch visible.

Stage 2: Buffer and standard preparation

Step	Action	Observation
6	Bring wash buffer concentrate to room temperature, mix to dissolve precipitated salts, and dilute with deionized water per protocol.	1X wash buffer prepared.
7	Reconstitute one vial of protein standard: add buffer without pipetting up and down, swirl and invert five times or vortex briefly, and let sit for at least 10 minutes.	Standard fully reconstituted.
8	Prepare a 1:2 serial dilution of the standard, adding diluent to each tube, adding standard to the first tube, mixing thoroughly, and changing tips between tubes.	Serial dilution series ready for plate loading.

Technician pouring deionized water into a bottle labeled "1X Wash Buffer" at the laboratory bench.

Lab bench showing the reconstituted protein standard vial alongside wash buffer bottles and other reagents.

Technician pipetting into a row of tubes held in a blue rack, with wash buffer and standard vials nearby.

Stage 3: Plate loading, incubation, and washing

Step	Action	Observation
1	Add standards, controls, and samples to labeled wells using a fresh tip for each addition; pre-dilute samples if required.	Plate loaded with calibration and test wells.
2	Confirm whether the kit protocol calls for sequential or co-incubation with the detector antibody (co-incubation demonstrated here).	Correct incubation approach selected.
3	Dilute the assay buffer and biotin conjugate as instructed, then add the diluted detector antibody to each well.	Detector antibody added to all wells.

4	Recognize that the detector antibody binds the antigen at a site distinct from the capture antibody, forming the sandwich complex.	Sandwich complex formation understood.
5	Seal the plate and incubate at the time and temperature specified in the protocol.	Plate incubated as specified.
6	Wash the plate (manually or with an automated washer): fill wells, soak, decant, repeat 3–5 times, and tap dry on paper towel without letting wells dry out.	Unbound reagents removed.
7	Dilute the streptavidin-HRP concentrate with assay buffer and mix gently.	Detection reagent prepared for the next phase.

Gloved hand holding a blue rack of labeled tubes (Std1–Std7, Background) while pipetting into strip tubes, with the 1X Wash Buffer bottle and ELISA plate on the bench.

Technician pipetting the diluted biotin conjugate into the ELISA plate wells, with the tube rack, labeled reagents, and waste container visible.

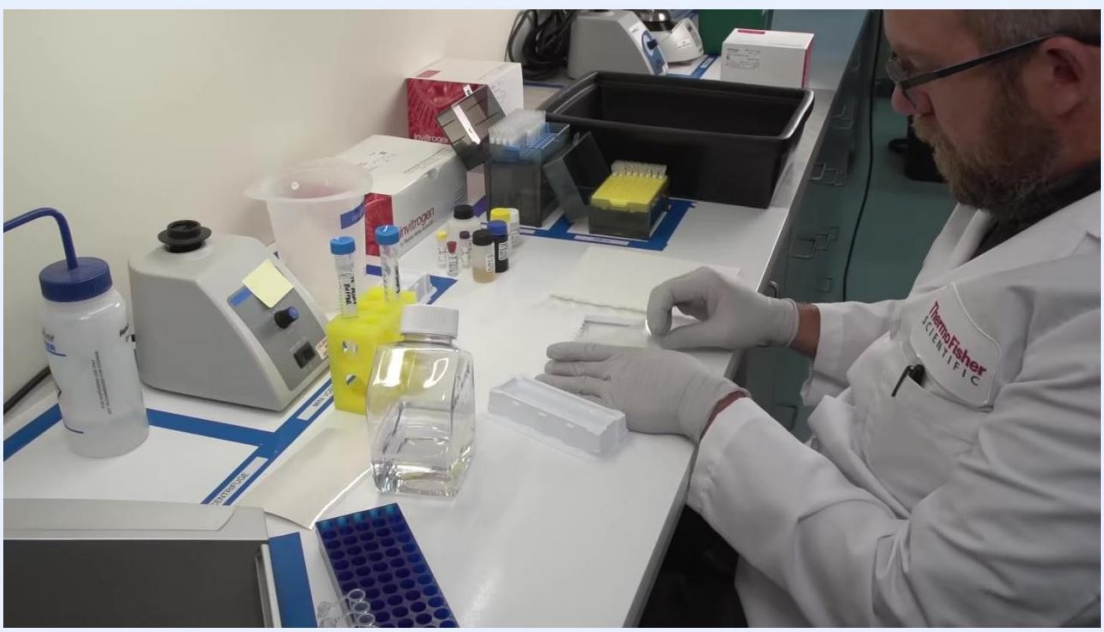
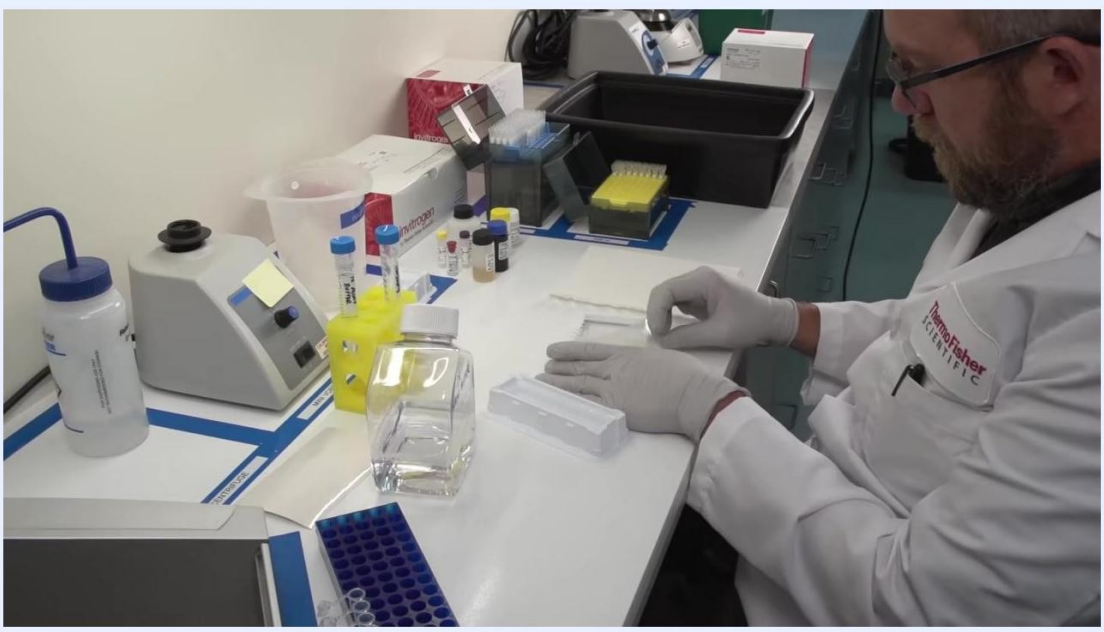


Diagram illustrating the sandwich ELISA principle: green capture antibodies bound to the plate, blue antigen, and blue detector antibody binding the antigen at a separate site.



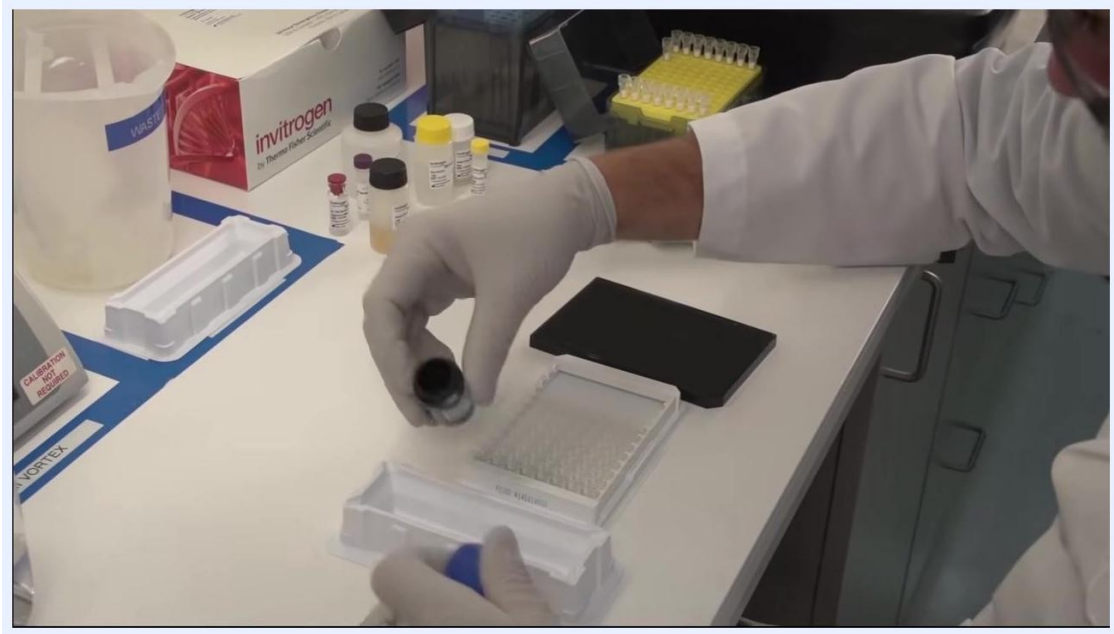
Technician manually washing the ELISA plate at the bench, with wash buffer, tube racks, and absorbent paper towel visible.

Gloved hands preparing the streptavidin-HRP conjugate by adding buffer to a labeled tube, with pipettes and the ELISA plate on the bench.

Stage 4: Signal development, stopping the reaction, and plate reading

Step	Action	Observation
1	Recognize that multiple streptavidin binding sites per biotin-labeled antibody produce enzymatic signal amplification.	Amplification mechanism understood.
2	Incubate the plate with streptavidin-HRP, covered, at the time and temperature specified.	Enzyme conjugate bound.
3	Wash the plate again (fill, soak, decant, tap dry) for the specified number of washes.	Excess enzyme removed.
4	Add chromogenic substrate to each well.	Substrate reacts with HRP to develop color.
5	Incubate at room temperature in the dark for 30 minutes, or as specified.	Color intensity proportional to protein concentration.
6	Add stop solution to each well to halt the enzymatic reaction.	Solution turns yellow, confirming reaction stop.

7	Measure absorbance at 450 nm (A450) using a plate reader and record values.	Absorbance data captured for analysis.
8	Use the standard curve from the standards to determine unknown sample concentrations.	Protein concentration in samples determined.



Technician adding the chromogenic substrate solution to the ELISA plate wells, with labeled reagents and pipettes on the bench.

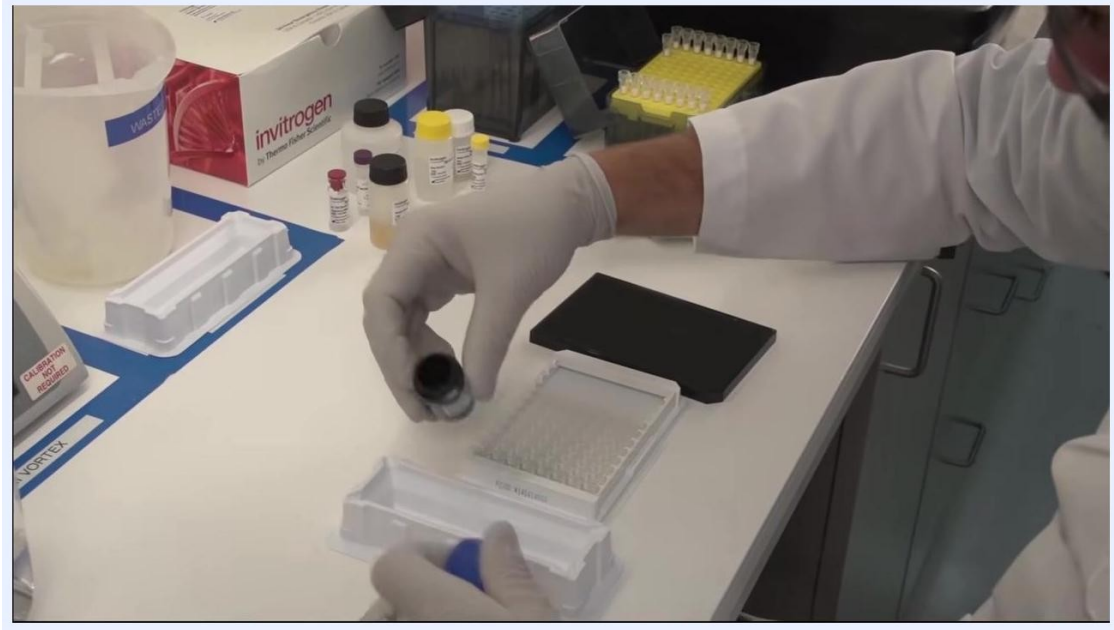
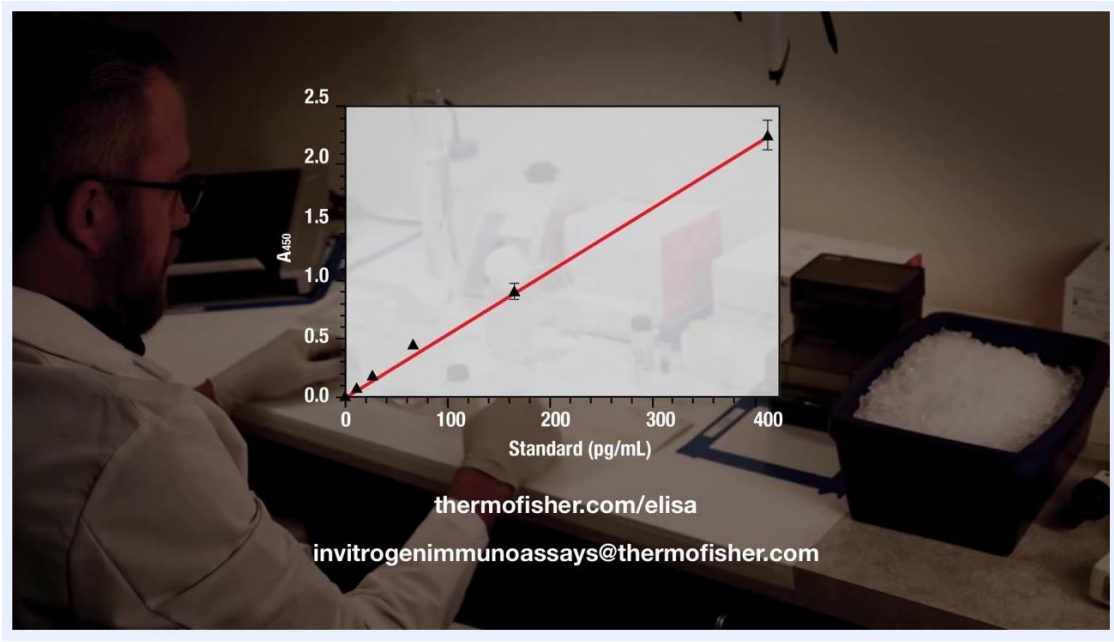
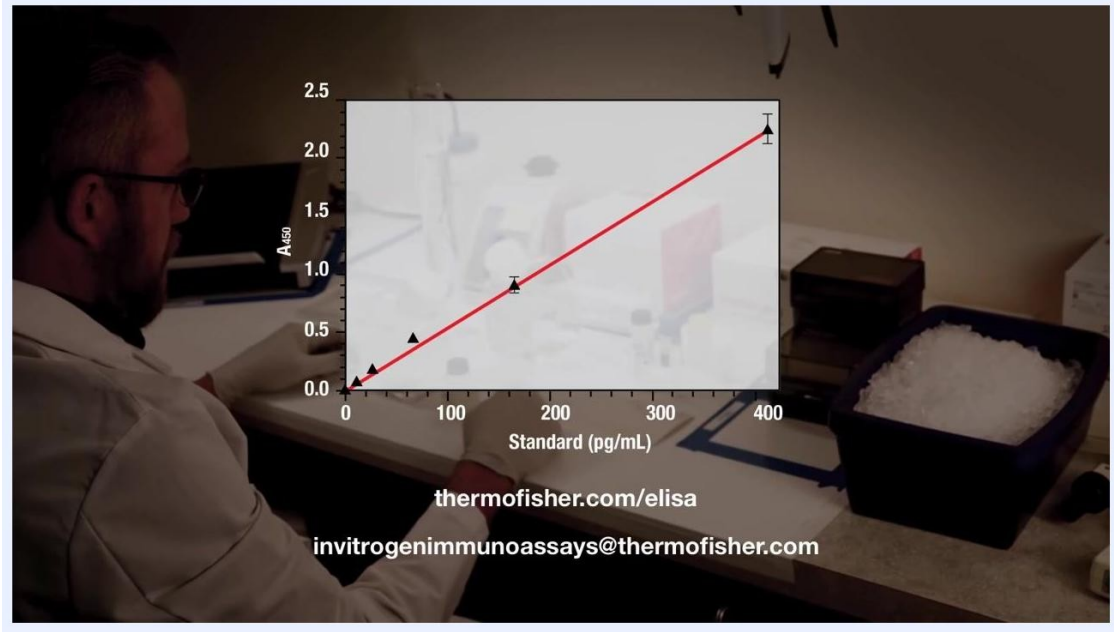


Diagram showing green capture antibodies, blue detector antibodies, and red streptavidin-HRP conjugates bound in a well, illustrating the enzymatic amplification principle.



Gloved hands holding the Stop Solution bottle, ready to add it to the ELISA plate, with other reagents and pipettes visible.



Technician at the lab bench with the ELISA plate and a graph overlay showing absorbance (A450) versus standard concentration (pg/mL), illustrating the linear standard curve, alongside technical support contact information.

Deviations

No protocol deviations are described in this procedure; each step is presented as following the kit-specific protocol as written. Where a laboratory deviates from

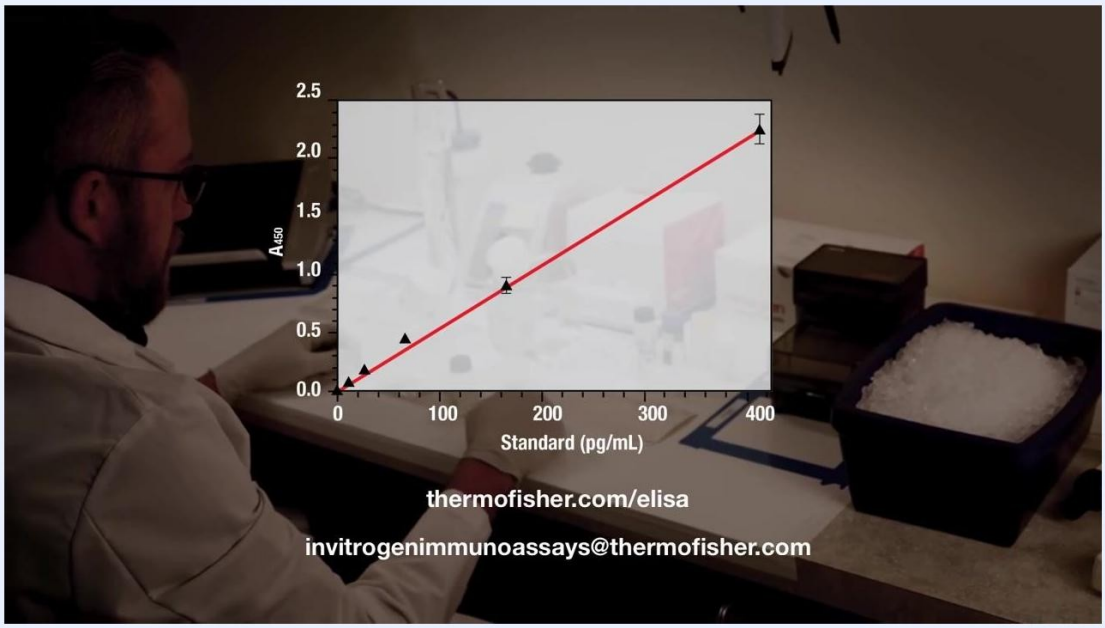
timing, temperature, dilution ratios, or wash cycle counts specified in its kit insert, the deviation, its impact on assay performance, and a determination of data validity should be documented here.

Acceptance summary

Formal acceptance criteria (e.g., standard curve R^2 threshold, %CV limits, control well ranges) are not specified in this procedure. Predefined acceptance criteria for each validation parameter in the Validation Design section should be compared against actual run data and documented here, including any limitations identified (for example, reliance on a single demonstrated run rather than replicate runs across operators, days, or lots).

Conclusion

Following the steps above prepares the Invitrogen ELISA kit reagents, loads the plate, develops and stops the enzymatic signal, and reads absorbance at 450 nm against a standard curve to determine sample protein concentration. This procedure demonstrates how to run an ELISA assay using the kit as intended, with routine controls consisting of the kit-supplied standards (for the calibration curve) and a background well; formal statistical validation (precision, accuracy, robustness testing) would need to be completed separately before the assay is declared validated for routine use. For further product information, visit thermofisher.com/elisa, or contact invitrogenimmunoassays@thermofisher.com for technical questions.



Thermo Fisher Scientific logo with associated brands: Thermo Scientific, Applied Biosystems, Invitrogen, Fisher Scientific, Unity Lab Services.