Reed® Biotech

VeriQuant™ Guide

USER GUIDE

This Guide is also applicable to both the 20 µL and 50 µL assay formats. To minimize pipetting errors and improve experimental success rates, new users are strongly recommended to use the 50 µL format.

Where Boundless Ideas Converge with Cutting-Edge Science

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Product Information

Product Description

The VeriQuant™ Immunoassay Kit is a qPCR-based assay designed for the in vitro quantitative detection of specific target proteins. The kit offers a broad dynamic range, high sensitivity, and requires only a minimal sample volume. The workflow is streamlined and eliminates the need for complex washing procedures.
Because this technology pushes the boundaries of sensitivity while enabling wash-free, shake-free, and low-sample-volume detection, special attention must be paid to pipetting accuracy and proper mixing throughout the assay procedure to ensure optimal experimental performance.

Principle of the Assay

Two antibodies recognizing distinct epitopes of the target protein are each conjugated with specific oligonucleotides. When both antibodies bind to the same target protein, the two oligonucleotides are brought into close proximity. Subsequently, Synthesis Enzymes ligate the oligonucleotides to form a template, which is amplified via qPCR using specific primers and TaqMan probes. Fluorescence signal intensity is negatively correlated with the cycle threshold (Ct) value, enabling high-specificity and high-sensitivity quantitative detection of the target protein.

Kit Components and Storage Conditions

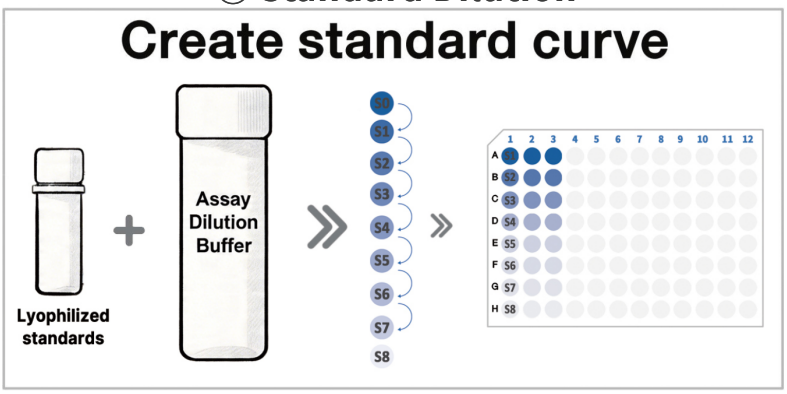
The VeriQuant™ Immunoassay Kit is shipped with a low-temperature phase-change material to maintain reagent stability during transportation.
Upon receipt, store the entire kit at −20°C.
Each kit contains all reagents required to perform the complete assay.

Required Materials Not Supplied

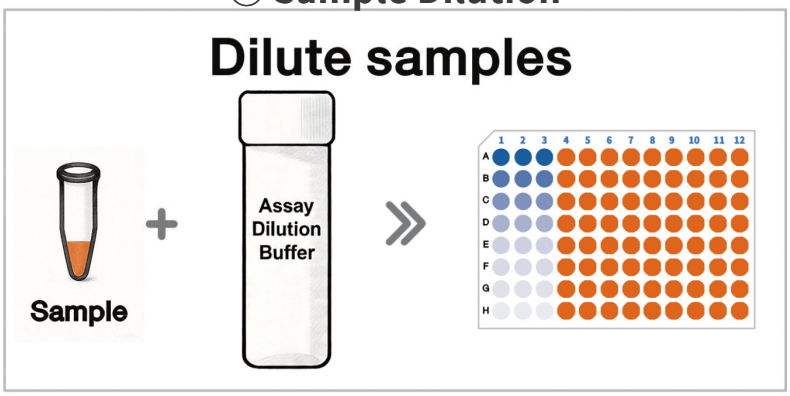
- Real-time quantitative PCR instrument
- Two pieces of 96-well 0.2 mL PCR plates (PCR 8-tube strips or single PCR tubes are acceptable alternatives)
- High-precision single-channel and multi-channel pipettes, centrifuge tubes, and low-retention pipette tips (0.5–10 µL, 2–20 µL, 20–200 µL, 200–1000 µL)
- Incubator
- Double-distilled water or deionized water
- Pressure-sensitive high-adhesion qPCR sealing film, sealing film scraper or roller
- 2 × 5 mL reagent reservoirs
- 96-well low-temperature metal ice block
- DNase/RNase/pyrogen-free 1.5 mL centrifuge tubes
- Microplate centrifuge

Experimental Workflow

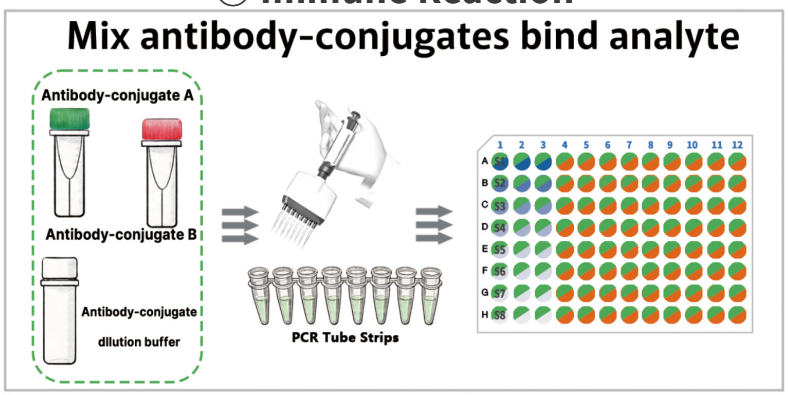
① Standard Dilution



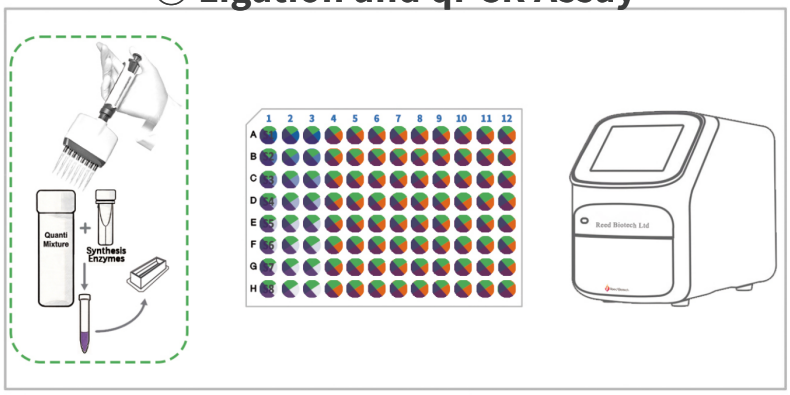
② Sample Dilution

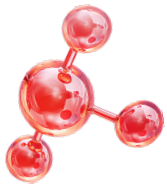


③ Immune Reaction



④ Ligation and qPCR Assay





Experiment Details

Precautions

• Wear gloves, use DNase/RNase/pyrogen-free plastic container and practice proper DNA handling techniques.
• Use a sealing film applicator or roller to ensure the fluorescent quantitative PCR sealing film is fully adhered to the PCR plate, preventing any evaporation or contamination.
• Use optimal pipetting techniques to minimize the CV value.
• At the start of the experiment, prepare two PCR plates, named PCR Plate 1 (for convenient use with a multichannel pipette, used for temporary storage of all prepared reagents) and PCR Plate 2 (the reaction plate for the formal experiment).
• Use a plate holder to secure PCR Plate 2 to prevent liquid splashing when opening the sealing film.
• It is recommended to run the experiment in triplicate so that outliers are easier to identify.
• Ensure the use of a PCR plate that is compatible with the qPCR instrument and module.
• Do not vortex the PCR plate.
• Dilution or mixing of reagents is critical to the experimental results. This can be done by carefully pipetting up and down 10 times, or by tapping the side of the plate against a solid object such as the palm of the hand or a laboratory bench.
• Before the reaction, use a microplate centrifuge to centrifuge briefly at low speed to collect residual liquid from the tube caps and walls down to the bottom of the tubes or plate. Avoid generating bubbles during operation. This step is very important; inadequate or incomplete mixing can affect baseline stability.

Reagent preparation

• Centrifuge reagent tubes before pipetting to ensure that all liquid is collected at the bottom of the tube.
• Synthesis Enzymes do not require thawing. Always keep Synthesis Enzymes at -20°C or on ice. Synthesis Enzymes are highly viscous; pipetting must be performed accurately and carefully, and must be taken to avoid generating bubbles.
• Except Synthesis Enzymes, all reagents should be thawed at room temperature.
• Keep Synthesis Enzymes and thawed reagents on ice during sample loading operations.
• During preparation, use a refrigerated 96-well low-temperature metal cooling block for PCR Plate 1 to keep all reagents at a low temperature. If a 96-well low-temperature metal cooling block is not available, please place PCR Plate 1 on ice.
• If any particulate substances is present in the samples, centrifuge or filter the samples before proceeding with the assay.

Dilution Procedure Instructions

• Good pipetting practices are critical for achieving optimal CV values when handling sample dilutions.
• Ensure that pipettes are calibrated. Use high-precision pipettes with appropriate volume ranges, such as 2 µL or 20 µL. The use of a multichannel pipette is highly recommended to facilitate experimental operations and minimize CV values.
• Use low-retention tips.
• The sealing film must be firmly attached to the PCR plate, especially along the plate edges, using a sealing film applicator or roller to ensure proper adhesion. This will prevent any liquid evaporation and avoid cross-contamination between wells during mixing.
• Use low-retention reagent reservoirs specifically designed for micro-volume pipetting, facilitating use with multichannel pipettes.

Creating Standard Curves Instructions

• Ensure that the calculation setup and procedures for reconstitution and serial dilution have been performed correctly.
• Ensure that the reconstitution of the standards has been performed correctly.
• Ensure thorough mixing at each step of the serial dilution, and change pipette tips for every step (discard after use).
• Check the standard curve data to identify any outliers. The default criteria for outliers are: any value with recovery outside 70%–130% of the standard value, or a CV (coefficient of variation) greater than 15% for replicate data.

Reagents Preparation

Reagent Thawing

Synthesis Enzymes are in liquid form and do not require thawing. Thaw all reagents at room temperature except Synthesis Enzymes. Keep Synthesis Enzymes (no thawing required) and all thawed reagents on ice during the entire experiment.

Sample Preparation

1.To minimize the matrix effect on the assay, all samples must be diluted 10-fold before testing. Add the appropriate volumes of sample and Assay Dilution Buffer to each well of PCR Plate 1. The final volume may be adjusted according to actual experimental needs.

Component	Volume
Test Sample	5 µL
Assay Dilution Buffer	45 µL

2.Mix well by gentle pipetting to homogenize the liquid in the test well.

Mix Antibody-Conjugates

Calculate the required volume of antibody-conjugate mixture based on the actual number of test wells and reaction volume. Adjust the amount proportionally according to the reaction system. To account for pipetting losses, the actual prepared volume should be at least 30% higher than the theoretical volume. For a 20 µL reaction system, add 2 µL of antibody-conjugate mixture per well. For a 50 µL reaction system, add 5 µL of antibody-conjugate mixture per well. Below is the recommended reaction setup based on the dosage for a 96-well plate.

1.Add the following components into a 1.5 mL centrifuge tube at a volume ratio of 1:1:100, and mix by inverting the tube up and down.

Component	20 µL Reaction System	50 µL Reaction System
Antibody-conjugate A	3 µL	7 µL
Antibody-conjugate B	3 µL	7 µL
Antibody-conjugate Dilution Buffer	300 µL	700 µL

2. Add the appropriate volume of the mixed antibody-conjugate mixture to each well of one column on PCR Plate 1. The volume should be determined according to the reaction system to facilitate operation with an 8-channel pipette.

For a 20 µL reaction system: Add ≥ 32 µL per well in the column (the theoretical volume for the 12 wells in the same row is 2 × 12 = 24 µL).

For a 50 µL reaction system: Add ≥ 80 µL per well in the column (the theoretical volume for the 12 wells in the same row is 5 × 12 = 60 µL).

Reconstitution of Standards

(Taking Mouse IL-6 as an example, the standard is provided as 1000 pg lyophilized powder)

1.Add 1 mL of Assay Dilution Buffer to reconstitute one vial of standard to a concentration of 1000 pg/mL. Mix thoroughly by inverting the vial up and down five times; do not vortex.
(Note: Do not mix by pipetting up and down, as the crystalline powder may adhere to the pipette tip. If the powder is not fully dissolved after a few minutes, repeat the inversion.)

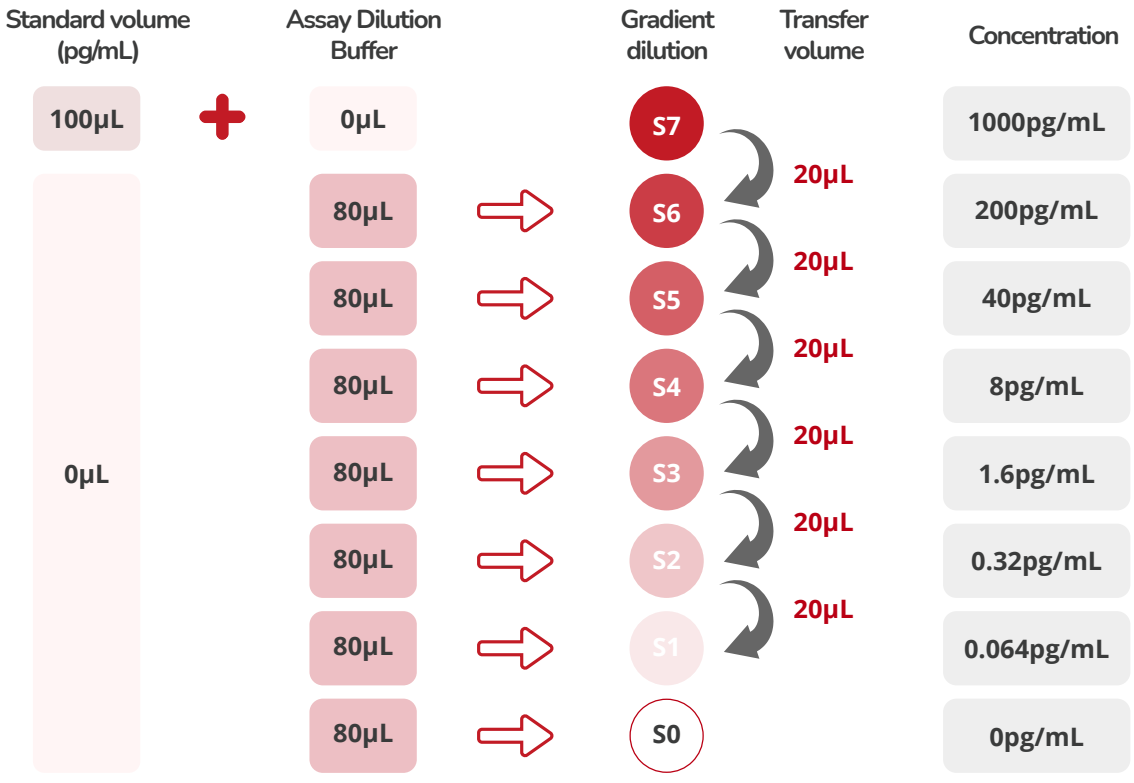
2.Stand at room temperature for 15 minutes.

Create Standard Curve

3.Prepare PCR Plate 1 (for temporary storage of all prepared working solutions to facilitate subsequent multichannel pipetting; standard curve dilutions must be performed within the same column. Keep PCR Plate 1 on a low-temperature metal ice block throughout the experiment).

4.Conduct **5-fold** serial dilution: Add 80 µL of Assay Dilution Buffer to each well **starting from the second well** of the standard column on PCR Plate 1. Pipette 100 µL of the 5000 pg/mL standard working solution into the first well (marked **S7**). Transfer 20 µL from S7 to the second well and mix to obtain **S6**(1000 pg/mL). Continue the dilution sequentially down to the penultimate well (**S1**). The last well serves as the blank control (**S0**) with no standard added. Prepare standard working solutions freshly right before use.

5.Press the sealing film evenly with a roller for complete sealing. Tap the side of the plate gently three times for thorough mixing, then centrifuge the plate at 3000 g for 1 minute using a microplate centrifuge.



Assay Procedures

A. Analyte Binding（Incubated at 37°C for 1 hour）

1. Using a multichannel pipette, transfer the appropriate volume of Antibody Conjugate Mixture and Standards or diluted Samples from PCR Plate 1 to the assay wells of PCR Plate 2 according to the selected reaction volume. The dispensing volumes for different reaction systems are listed in the table below.

Reaction system	20 µL reaction system	50 µL reaction system
Antibody-conjugate mixture	2 µL	5 µL
Standard or Diluted Sample	2 µL	5 µL

2. Seal PCR Plate 2 with a qPCR sealing film. Gently tap the side of the plate three times with the palm of your hand to ensure thorough mixing. Centrifuge at 3,000 × g for 1 minute.

3. Incubate PCR Plate 2 at room temperature for 1 hour. Except during mixing or centrifugation steps, keep PCR Plate 2 stationary on a plate rack.

B.qPCR Amplification

1. Pre-mix qPCR reaction mixture before the completion of Step A: Add 25 µL of **Synthesis Enzymes** into the **Quanti Mixture**(volume ratio of Synthesis Enzymes to Quanti Mixture = 1:200). Mix by inverting the tube five times. Transfer the mixture to a reagent reservoir for convenient multi-channel pipetting if needed.

2. Add 40 µL of the prepared qPCR reaction mixture to each well of PCR Plate 2. Mix gently and avoid bubble formation.

Reaction system	20 µL reaction system	50 µL reaction system
qPCR Reaction Mixture	16 µL	40 µL

3. Seal the plate tightly with qPCR sealing film and press firmly with a roller.

4. Tap the plate side gently to mix, then centrifuge at 3000 g for 1 minute.

5. Create a new protocol on the qPCR instrument with the following settings.

6.Enter the real-time quantitative PCR (qPCR) instrument parameters.

Item	Parameter
Assay Type	Standard Curve /Quantitation Standard Curve
Reporter Dye	FAM
Quencher	NFQ-MGB *
Reference Dye	None
Sample Type for all wells	Unknown
Threshold	0.2
Baseline	15
Benchmark	3-15

* If the corresponding option is unavailable on your instrument, select "None" or "Non-fluorescent".

7.Select the appropriate cycling conditions according to the module type and run the qPCR plate.

Step	Temp(°C)*	Time (50μL)	Time (20μL)	Stage
Ligation	25	20min	20min	Hold
Inactivation	95	2min	2min	Hold
Denaturation	95	15 s	5 s	40 cycles
Annealing/extension	60	1min	1min	

* Ramp rate: 1.6 °C/s

8.Save the experiment as a template and run the template.

Note: Reuse the saved template for subsequent assays.

Data Analysis

1. Save the assay data in .ed, .sds or .csv format.

2. Calculate the average Ct value for duplicate wells of each standard. Use the 4-parameter logistic (4PL) curve fitting tool (available on www.reedbiotech.com) to generate a standard curve on a double logarithmic coordinate system, with standard concentration on the X-axis and average Ct value on the Y-axis. Calculate sample concentrations by substituting sample average Ct values into the standard curve equation.

Troubleshooting

Observation	Possible cause	Recommended action
No Ct values in data file	qPCR software was not set up properly	Make sure that all 96 wells are designated as unknown.
		Make sure that the parameters including FAM, ROX passive was set.
		Make sure the camera collected is at the last cycle point.
Poor standard curve (poor recovery)	Improper serial dilution	Verify that amounts of Assay Dilution Buffer and recombinant protein is correct for each well.
		Verify that the range of dilutions are within the recommended range in the kit protocol.
	Contamination from well to well.	Make sure that the plates are sealed tightly so that no spillage happens during mixing and plate centrifugation.
		Make sure to change pipette tips in between wells or samples.
Poor standard curve (high CV)	Improper pipetting technique	Verify that pipettes are calibrated.
		Use low-retention filtered tips, especially for low-volume transfers.
		Minimize bubble formation during repeated aspiration/ dispensing.
		Follow proper pipetting practices (dispense against the well wall and visually inspect volume accuracy).
		Use a multichannel pipette whenever possible.
		Ensure tips are firmly attached and aspirated volumes are correct.
		Avoid reverse pipetting for microliter-scale transfers.
		If consistency is poor at very low volumes (e.g., 2 µL), increase the transfer volume to 5 µL if feasible.
	Improper mixing	Confirm the PCR plate is properly sealed using a plate sealer.
		Thoroughly mix after each reagent addition (e.g., pipette up/down 10 times or tap the plate sufficiently to move liquid across the well).
		Centrifuge the PCR plate after mixing to bring all reagents to the bottom of the wells.
	Evaporation during low-volume handling	Minimize preparation time and avoid prolonged exposure of microliter-volume liquids by using PCR plates or equivalent methods.
	Insufficient replicates	Perform samples in triplicate to facilitate identification of outliers.
Poor standard curve (high low end CV) High CV only at the low end of the curve but not the linear portion of the curve	The assay is at the limit of sensitivity	Acceptable data is when the CV is less than 20%, so data cannot be reliable in this range.