RayBiotech
Empowering your proteomics

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NexaTag™ Human S100A4 qIPCR ELISA Kit

Catalog Number: BIQH-S100A4

Manual
Last Revised: 06/19/2025

Introduction

The RayBio® NexaTag™ quantitative immuno-PCR ELISA (previously known as BIQ-ELISA) is an innovative protein detection method that merges the specificity of sandwich-based ELISA, the sensitivity of real-time PCR, and the easy manipulation of magnetic microspheres. In this system, the capture antibody (CAB) is biotin-labelled and pre-absorbed onto streptavidin-coated beads (CAB-Biotin-SA-Beads), while the detection antibody (DAB) is conjugated with a unique oligonucleotide acid (ONA) barcode for signal amplification (DAB-ONA).

The procedure is straightforward and a total of four hours is required to complete the assay. Part 1: The formation and purification of the immunocomplex (ONA-DAB-Antigen-CAB-Biotin-SA-Beads). Samples are incubated with DAB-ONA and CAB-Biotin-SA-Beads in a supplied 96-well plate. In the presence of the target protein, the DAB-ONA binds to the target and attaches to the CAB-Biotin-SA-Beads via the biotin-streptavidin interaction and the unbound proteins are removed through washing with a magnetic automated washer. Part 2: PCR amplification. The bound immunocomplex will be released from the beads and transferred as the template for qPCR. Primers and PCR master mix are added to the wells and data will be collected using qPCR. Ct values obtained from the qPCR are then used to calculate the amount of antigen contained in each sample, where lower Ct values indicate a higher concentration of antigen. In a real experiment, a set of target protein standards with known concentrations will be run simultaneously to generate a standard curve for quantifying unknown samples.

The RayBio® NexaTag™ qIPCR ELISA technology represents a significant advancement in protein detection assays, providing an ultrasensitive and highly specific method for quantifying proteins in biological samples. This technology's ability to combine multiple advantages of existing methods into a single, easy-to-use platform makes it a powerful tool for research community. The RayBio® NexaTag™ qIPCR ELISA kits can be used for the quantitative measurement of proteins in various sample types including serum, plasma, and cell culture supernatants with femtogram per milliliter detection sensitivity.

Storage / Stability

Upon receiving the kit, store 2X PCR Master Mix, Primer Mix, and Human S100A4 Standard at -20°C. Store other components at 4°C. If stored at the proper temperature, the kit may be stored for up to 12 months from the date of shipment. Avoid repeated freeze-thaw cycles.

Kit Components

Name	Catalog #	Size / Qty	Storage After Preparation
96-well V-bottom plate	BIQ-VP	1 plate	-
96-well Plate Film	BIQ-FILM	1 piece	-
96-well PCR Plate*	BIQ-PP	1 plate	-
1X Assay Diluent	BIQ-AD	1 bottle (25 mL)	4°C
20X Wash Buffer Concentrate	BIQ-WB	1 bottle (30 mL)	4°C
Human S100A4 Standard	BIQH-S100A4-STD	1 vial	-20°C or -80°C
Human S100A4 Antibody Coated Beads	BIQH-S100A4-Bead	1 vial (0.6 mL)	4°C
Oligonucleotide Conjugated Antibody	BIQH-S100A4-OAC	1 vial (10 µL)	4°C
1X Elution Buffer	BIQ-ELU	1 vial (5 mL)	4°C
2X PCR Master Mix	BIQ-MM	1 vial (1.05 mL)	-20°C
Primer Mix	BIQ-PM	1 vial (lyophilized)	-20°C
PCR Plate Film	BIQ-SEAL	1 piece	-

*The PCR plate used is a 0.2 mL, non-skirted 96-well plate (Thermo Fisher, cat. no. AB0600). Please ensure compatibility with your PCR machine prior to purchase. For additional information contact technical support (techsupport@raybiotech.com).

Additional Materials Required

- Real-time PCR instrument, Bio-Rad or QuantStudio™ recommended
- Orbital 96-well plate shaker (with ability to reach 700 rpm)
- Microplate Washer (BioTek 405 TS Washer recommended) and a compatible 96-well V-bottom plate magnetic stand (ThermoFisher AM10050 recommended)
- Precision pipettes to deliver 1 µL to 1 mL volumes
- Pipette tips (barrier tips are recommended to reduce contamination)
- Adjustable 1-25 mL pipettes for reagent preparation
- Centrifuge that can spin 96 well plates at 500 g
- Nuclease-free water or deionized water
- 1.5 mL, 2 mL, or 5 mL microcentrifuge tubes

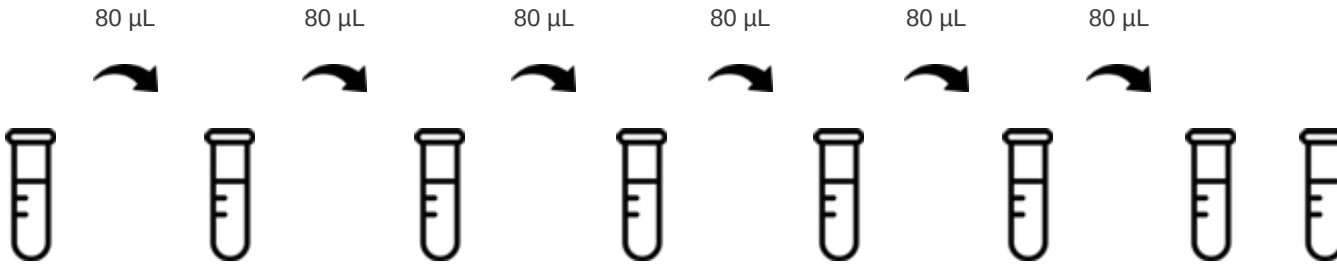
Reagent and Sample Preparation

1. Briefly centrifuge the Primer Mix tube and then reconstitute the powder with 0.55 mL nuclease-free water.
2. Prepare 1X Wash Buffer. If the 20X Wash Buffer Concentrate contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 25 mL 20X Wash Buffer Concentrate with deionized or distilled water to yield 500 mL of 1X Wash Buffer.
3. Prime the Microplate Washer system with 1X Wash Buffer.
4. Dilute samples in 1X Assay Diluent. For high-fold dilutions, first dilute samples in PBS and then dilute in 1X Assay Diluent. Suggested dilution for normal serum/plasma: 50- to 500-fold.

   Note: the levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

Standard Preparation

Preparation of Human S100A4 Standard: Briefly spin the Human S100A4 Standard Vial. Add 500 µL 1X Assay Diluent to reconstitute the Standard Solution (Std 1). Gently mix the powder to allow it to dissolve thoroughly. Pipette 160 µL 1X Assay Diluent into each of 7 additional tubes. Use the reconstituted Standard Solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1X Assay Diluent serves as the blank control.



Vial labels	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Cont
Diluent volume	500 µL	160 µL	160 µL	160 µL	160 µL	160 µL	160 µL	160 µ
Final concentration in assay (pg/mL)	1000000	333333.33	111111.11	37037.04	12345.68	4115.23	1371.74	0

Assay Procedure

1. Place all reagents on ice to thaw before use. PCR Master Mix and Primer Mix should always be kept on ice after thawing. It is recommended to run all standards and samples in duplicates or triplicates.
2. Label the V-bottom 96-well plate as appropriate for your experiment.
3. Add 6 µL Oligonucleotide Conjugated Antibody into the vial of Human S100A4 antibody coated beads. Vortex the vial for 30 seconds to fully mix the components. Aliquot 5 µL to each well.

   Note: The Assay Diluent is viscous, reverse pipetting may be used to avoid producing bubbles.

4. Add 5 µL of each standard and sample into appropriate wells so that the total volume in each well is 10 µL. Seal the plate using the 96-well Plate Film.
5. Briefly spin the sealed plate at 500 g to pool all liquid to the bottom of the wells.
6. Place the plate on an orbital plate shaker. Shake at 700 rpm at room temperature for 2.5 hours.
7. Briefly spin the sealed plate at 500 g to pool all liquid to the bottom of the wells. Carefully remove the film.
8. Add 40 µL Assay Diluent to each well to bring the volume to 50 µL per well. Note: Bringing the volume up to 50 µL here is required for optimal washing in the next step.
9. Wash the plate 5 times with 200 µL 1X Wash Buffer on the magnet with the Microplate Washer.

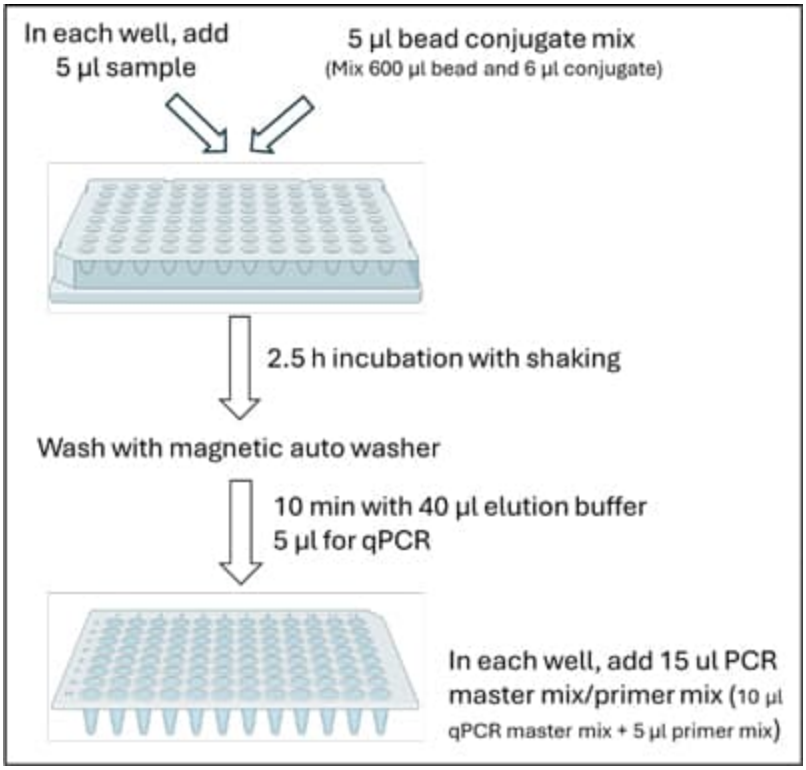
   Note: A successful wash step is important to get a good result. The microplate washer should be optimized to ensure minimum bead loss and an even wash across the plate. See Appendix for a reference program used on BioTek 405 TS Washer.
10. Add 40 µL 1X Elution Buffer to each well, use the microplate washer to shake the plate for 15 seconds without the magnet. Then, incubate at room temperature for 10 minutes. During this incubation step, complete Step 11.
11. Prepare the PCR/Primer master mix by mixing 2 volumes of the 2X PCR Master Mix with 1 volume of Primer Mix (see note below). Pipette 15 µL of the PCR/Primer master mix into each well of the PCR plate.

    Note: The volume depends on the number of wells used, 15 µL PCR/Primer master mix is required for each well. To ensure there is enough PCR/Primer master mix, we recommend preparing at least two extra wells worth of volume. For example, for 24 wells, 260 µL 2X PCR Master Mix should be mixed with 130 µL Primer Mix.
12. After the 10-minute incubation, place the V-bottom plate on the magnetic stand and incubate at room temperature for 2 minutes to let the beads settle down.
13. Transfer 5 µL of the supernatant from each well to the corresponding well containing 15 µL PCR/Primer mix in the PCR plate so that the total volume in each well is 20 µL. Pipette thoroughly to mix (at least 3 times up and down).
14. Cover the plate with the supplied PCR Plate Film, taking care to ensure the film is completely and evenly pressed onto the plate, creating an airtight seal around each well of the plate.
15. Centrifuge the sealed PCR plate at 500 g for 1 minute to pool all liquid to the bottom of the wells.
16. Place the plate into a real-time PCR instrument using a SYBR Green I compatible wavelength for detection with the following settings:

Steps	Temperature (°C)	Time (sec)
Initial denature	95	300
40 cycles	95	15
40 cycles	60	20

Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed.
2. Add 5 µL of the bead and conjugate mix, and 5 µL of each standard and sample to each well. Incubate for 2.5 hours at room temperature while shaking.
3. Add 40 µL Assay Diluent and wash the plate using a Microplate Washer.
4. Add 40 µL Elution buffer to each well. Incubate for 10 minutes at room temperature.
5. Prepare PCR/Primer Master Mix and pipette 15 µL into each well of the PCR plate.
6. Transfer 5 µL supernatant from V-bottom plate to the PCR plate.
7. Run real-time PCR.



Calculation of Results

The primary data output of the NexaTag™ qIPCR ELISA kit is Ct values. These values represent the number of cycles required for a sample to pass a fluorescence threshold. As the DNA is amplified, additional fluorescent signals are produced, with each cycle resulting in an approximate doubling of the DNA. Therefore, higher levels of DNA (directly related to the amount of antigen in the sample) result in lower Ct values.

Remove obvious outliers from the results if present. Calculate the mean Ct for each set of triplicate standards, controls and samples. Subtract the Ct value of each sample from the control to obtain the difference between the control and sample (Delta Ct). Plot the values of the standards on a graph using a log scale for concentration on the x axis. This graph is the quickest way to visualize results, although not the most accurate. If this method is used the concentration of unknown samples can be estimated using a logarithmic line of best fit.

The line of best fit will have an equation y = k(x)+b, where y is the Delta Ct value and x is the concentration. To calculate the concentration of the unknown sample from the standard curve, this can be entered into Excel in the following format:

Sample concentration = $10^{(y - b / k)}$

Where y is the Delta Ct obtained during the assay, and b and k are obtained from the line of best fit.

To obtain the final concentration of the sample, multiply the calculated concentration by the dilution factor used. Be sure to account for the 2-fold dilution that occurs from mixing the sample with the beads.

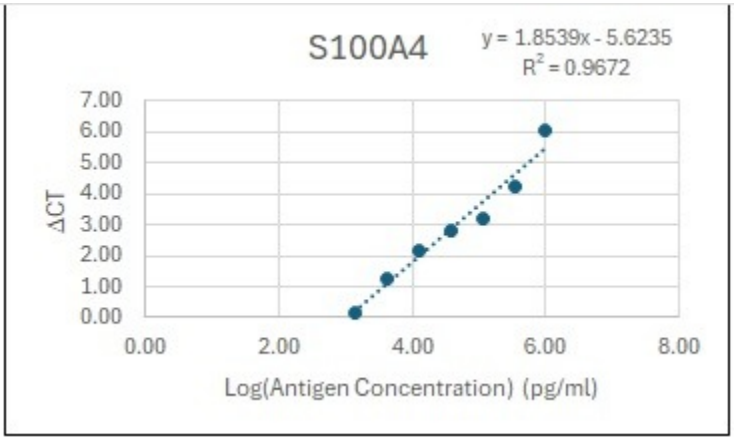
Ex: If you diluted your sample 10-fold, the final dilution factor would be 20 (10 x 2).

Typical Data

Standard Curve

These standard curves are for demonstration only. A standard curve must be run with each assay.

Conc (pg/ml)	Ct	ΔCt
1000000	28.51	6.06
333333.33	30.30	4.27
111111.11	31.34	3.23
37037.04	31.77	2.80
12345.68	32.42	2.15
4115.23	33.34	1.23
1371.74	34.38	0.19
0	34.57	



Sensitivity

The minimum detectable concentration of human S100A4 is 112 pg/ml.

Spiking and Recovery

The spiking recovery rate of human S100A4 in diverse sample types are listed below:

Sample Type	Recovery	Range
	116%	106-130%

Reproducibility

Intra-Assay: CV <10%

Inter-Assay: CV <15%

Specificity

The antibodies used in the kit are specific to its own protein.

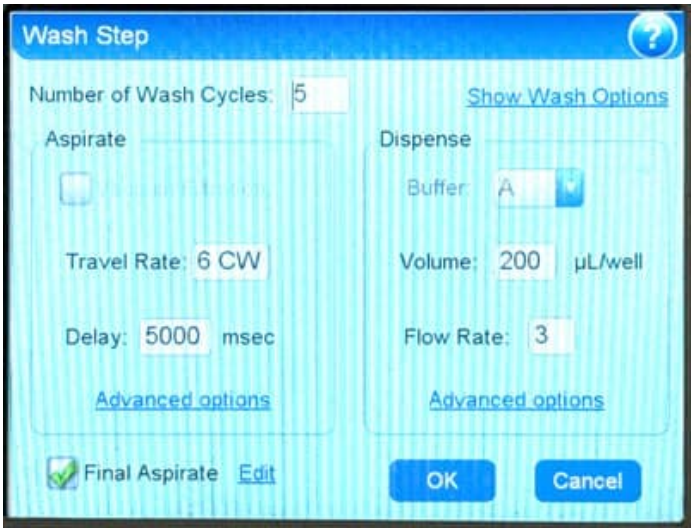
Troubleshooting Guide

Problem	Cause	Solution
Low signal in samples	• Sample concentration is too low • Improper preparation of detection antibody • Too brief incubation times • Inadequate reagent volumes or improper dilution • Beads were removed during wash	• Increase sample concentration • Briefly spin down vials before opening • Dissolve the powder thoroughly • Ensure sufficient incubation time; assay procedure step 3 may be done overnight. Check pipettes and ensure correct preparation. • Optimize the microplate washer
High signal in samples	Sample concentration is too high	Reduce sample concentration
Large CV	• Uneven pipetting • Bubbles present in wells • Uneven bead loss during wash	• Check pipettes • Centrifuge the plate before PCR • Optimize the microplate washer
High background	• Plate is insufficiently washed • Contaminated wash buffer	• Review the manual for proper wash. Ensure that all ports are unobstructed. • Make fresh wash buffer • Check run parameters and calibrate instrument
Low sensitivity	• Improper preparation of the standards • Beads were removed during wash • Oligo was not eluted • PCR CV was too high	• Prepare the standards following the protocol in this manual • Optimize the microplate washer • Shake and then incubate the beads in elution buffer for 10 minutes • Check run parameters and calibrate instrument

Appendix

Wash program for BioTek 405 TS Washer with Magnetic-Ring Stand (AM10050, ThermoFisher)

1. Soak: 2 minutes to let the beads settle down

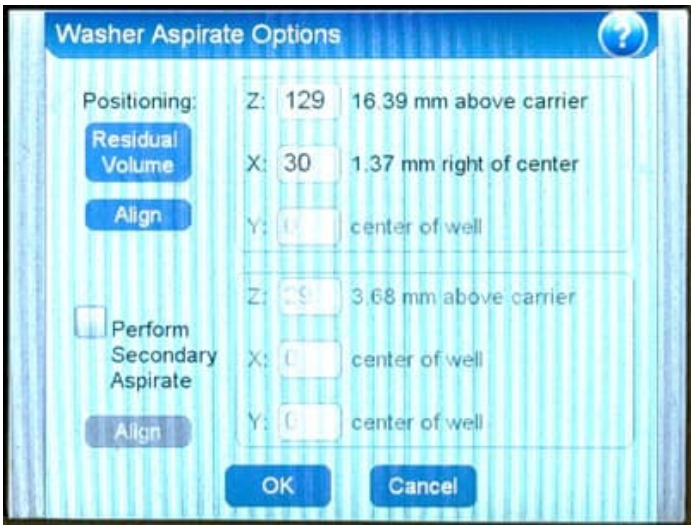


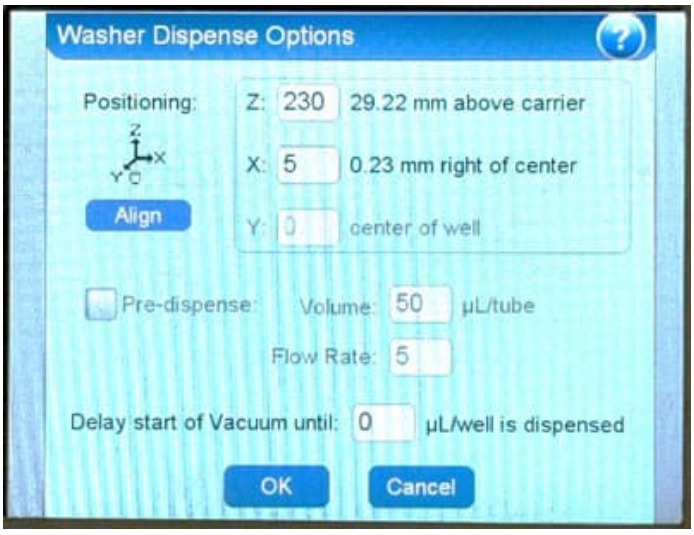
2. Wash-Aspiration: Travel Rate: 6 CW, Delay: 5 s, Alignment: X: 30, Z: 129

   Wash-Dispense: 200 µL wash buffer, Flow Rate: 3, Alignment: X: 5, Z: 230

   Wash-Cycle: 5

   Wash-Final Aspiration: Travel Rate: 6 CW, Delay: 5 s, Alignment: X: 30, Z: 129





Note: To ensure the buffer used is the 1X Wash Buffer, the Microplate Washer should be primed (250 mL) before washing the plate. Prime and one round of wash should consume 350 mL 1X Wash Buffer.

Note: After final aspiration, the plate should be dry. If there is liquid, the program may be optimized to improve evacuation.