RayBio®
IQELISA Conversion Kit

User Manual

RayBio® IQELISA Conversion Kit Protocol

(Cat#: IQC-CON-1)

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I. INTRODUCTION

The RayBio® **I**mmuno **Q**unatitative **E**nzyme **L**inked **I**mumuno**S**orbent **A**ssay (IQELISA) is an innovative new assay that combines the specificity and ease of use of an ELISA with the of real-time PCR. This results in an assay that is simultaneously familiar while also providing more sensitivity, typically, with 10-100x higher sensitivity when compared to a traditional ELISA. This conversion kit provides everything necessary to convert a standard ELISA assay into an IQELISA assay.

The majority of the steps of the ELISA remain unchanged, standards and samples are pipetted into wells, washed, the detection antibody is added, and excess antibody is washed away. After this step the normal detection molecule, generally Streptavidin-Horse Radish Peroxidase, is substituted for the IQELISA conversion reagents. The bound IQELISA detection molecules are eluted and transferred to a PCR plate or tubes. Primers and a PCR master mix are added to the wells and data is collected using qPCR. A C_t value is obtained from the qPCR data which is then used to calculate the amount of antigen contained in each sample, where lower C_q values indicate a higher concentration of antigen.

II. REAGENTS

1. IQ Conversion Reagent 1 (Item A): 1.3mL of a 20x concentrate
2. IQ Conversion Reagent 2 (Item B): 1.3mL of a 20x concentrate
3. PCR Primers (Item C): 200µL vial
4. PCR Master Mix (Item D): 1.3mL vial
5. Wash Buffer (Item E): 10ml vial of 10x concentrated buffer
6. Elution Buffer (Item F): 10mL vial of a 2x concentrated buffer

III. STORAGE

May be stored for up to 6 months at 2º to 8ºC from the date of shipment.
Opened reagents may be stored for up to 1 month at 2º to 8ºC.
Note: the kit can be used within one year if the whole kit is stored at -20 ºC.
Avoid repeated freeze-thaw cycles.

IV. ADDITIONAL MATERIALS REQUIRED

1 Real-time PCR instrument, Bio-Rad recommended
2 Precision pipettes to deliver 2 μl to 1 ml volumes.
3 Adjustable 1-25 ml pipettes for reagent preparation.
4 Absorbent paper.
5 Distilled or deionized water.
6 Log-log graph paper or computer and software for data analysis.
7 Tubes to prepare standard or sample dilutions.
8 Heating block or oven capable of 80^{O}C
9 PCR Plates or tubes
10 An ELISA kit including samples, standards, and other materials required for the assay.

V. REAGENT PREPARATION

1. Bring buffers and IQ detection reagents to room temperature (18 - 25°C) before use. PCR master mix (Item D) and Primer solution (Item C) should be kept at 4^{O}C at all times.

2. Wash Buffer (Item E) should be transferred to a 15mL tube and diluted with 9mL of deionized or distilled water for every 1mL of 10x concentrate used before use.

3. Elution buffer should be transferred to a 50mL conical tube and diluted with 10mL of distilled water.

VI. ASSAY PROCEDURE:

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run in triplicate.

2. Preform the ELISA assay as recommended by the manufacture through the detection step using the detection antibody supplied with the ELISA kit. Use the manufacture supplied wash buffer to wash as recommended.

3. Add 100 µl of prepared IQ Conversion Reagent (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

4. Wash each well 3x using Wash Buffer (Item E) by adding 100µL to each well. Wash by filling each well with Final Wash Buffer using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add 100µL Elution Buffer (Item F) into each well. The buffer may be heated to 80^{o}C prior to adding it into the wells. This helps release the antibodies from the well. If the buffer is heated take care to ensure that an equal amount of liquid is drawn into the pipette for each well. The increased temperature can result in uneven pipetting and care should be taken to ensure even distribution of the buffer into each well.

6. Cover the wells with plastic wrap or an adhesive film to prevent evaporation and incubate at 80^{o}C for 1 hour.

7. Remove the samples from the heat and allow the plate to cool to room temperature (about 30 minutes).

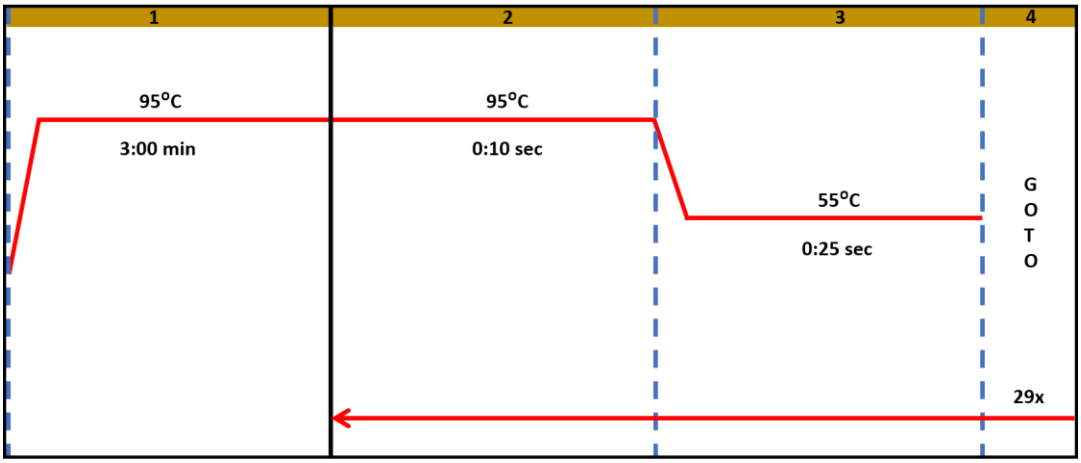
8. Transfer 2-10µL of the solution from each well of the microtiter plate into its own well in the PCR plate. If less than 10µL is transferred add deionized water to each well to bring the final volume in each well up to 10µL

9. Immediately before adding to the plate, mix 1µL of primers (Item c) for each well to be assayed into a fresh Eppendorf tube. To the same tube add 10µL of PCR Master Mix (Item D) and mix well.

10. Add 10µL of prepared PCR Master Mix to each well and mix thoroughly with a pipette (at least 3x up and down).

11. Cover the plate or tubes with an adhesive film or caps suitable of qPCR.

12. Place the samples into a real-time PCR instrument using a SYBR compatible wave length for detection with the following settings for cycling
    1. 3 minute activation at 95^{O}C
    2. 10 seconds 95^{O}C denaturation
    3. 25 seconds 55^{O}C annealing/extension
    4. Repeat steps 2 and 3 29x



VII. ASSAY PROCEDURE SUMMARY

1. Prepare all reagents as instructed.

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2. Perform ELISA as recommended by the manufacturer until Detection Antibodies have been added

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3. Add IQELISA Conversion Reagents

⇩

4. Wash the wells

⇩

5. Elute antibodies from the well and transfer to PCR compatible plate/tubes

⇩

6. Add master mix and Run real-time PCR

VIII. CALCULATION OF RESULTS

The primary data output of the IQELISA kit is C_q values. These values represent the number of cycles required for a sample to pass a fluorescence threshold. As the DNA is amplified additional fluorescent signal is 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 C_t values.

Calculate the mean C_q for each set of triplicate standards, controls and samples. Subtract the C_q value of each sample from the control to obtain the difference between the control and sample. Plot the values of the standards on a graph using a log scale for concentration on the x axis, and calculate a

standard curve using a log based line of best fit. A log based line of best fit should be used because C_q is a log based data.

The line of best fit will have an equation y = mln(x)+b, where y is the Delta C_q value and x is the concentration. It may be helpful to use 5 significant figures for m and b to minimize rounding errors. To calculate the concentration of unknown sample this can be entered into excel in the following format

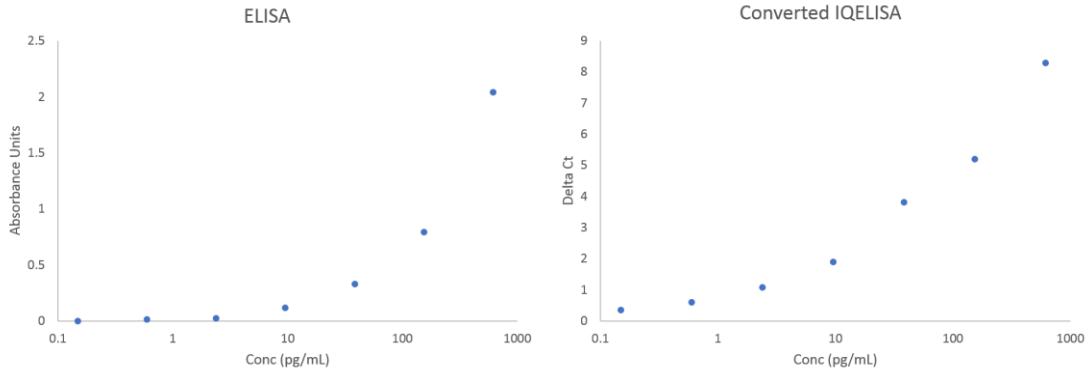
=EXP((y-b)/m))

Where y is the Delta C_q obtained during the assay, and b and m are obtained from the line of best fit

A data analysis tool for IQELISA is also available on the RayBiotech website on the IQELISA product page. This data analysis kit will calculate the results using a log/log conversion, as well as identify any possible outliers.

A. TYPICAL DATA

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



X. TROUBLESHOOTING GUIDE

Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting	1. Check pipettes
	2. Improper standard dilution	2. Ensure a brief spin of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1.Too brief incubation times	1. Ensure sufficient incubation time; assay procedure step 2 may change to overnight
	2. Inadequate reagent volumes or improper dilution	2. Check pipettes and ensure correct preparation
	3. Poor Elution	3. Heat buffer before adding to plate
3. Large CV	1. Uneven Pipetting	1. Check Pipettes
	2. Bubbles present in wells	2. Lightly tap or use pipette tip to dislodge from bottom of well
4. High background	1. Plate is insufficiently washed	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	2. Contaminated wash Buffer	2. Make fresh wash Buffer
	3. Improper Tm	3. Check run parameters and calibrate instrument
5. Low sensitivity	1. Improper storage of the Conversion Kit or ELISA	1. Store Kits as recommended
	2. Improper Tm	2. Check run parameters and calibrate instrument

This product is for research use only.