RayBio® Human PDGF-BB IQELISA Kit

User Manual

RayBio® Human PDGF-BB IQELISA Kit Protocol

(Cat#: IQH-PDGFBB)
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Tel:(Toll Free)1-888-494-8555 or 770-729-2992; Fax:770-206-2393
Web: www.raybiotech.com Email: info@raybiotech.com

RayBiotech, Inc.

RayBio® Human PDGF-BB IQELISA Kit Protocol

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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 sensitivity of real-time PCR. This results in an assay that is simultaneously familiar and cutting edge and enables the use of lower sample volumes while also providing more sensitivity. The RayBio® Human PDGF-BB IQELISA kit is a modified ELISA assay with high sensitivity qPCR readout for the quantitative measurement of human PDGF-BB in serum, plasma, and cell culture supernatants. This assay employs an antibody specific for human PDGF-BB coated on a 96-well PCR plate. Standards and samples are pipetted into the wells and PDGF-BB present in a sample is bound to the wells by the immobilized antibody. The wells are washed and a detection affinity molecule is added to the plates. After washing away unbound detection affinity molecule, primers and a PCR master mix are added to the wells and data is collected using qPCR. C_t values obtained from the qPCR are then used to calculate the amount of antigen contained in each sample, where lower C_t values indicate a higher concentration of antigen.

II. REAGENTS

1. PDGF-BB Microplate (Item A): 96 well PCR plate coated with anti-human PDGF-BB.
2. Wash Buffer I Concentrate (20x) (Item B): 25 ml of 20x concentrated solution.
3. Standards (Item C): 2 vials of recombinant human PDGF-BB.
4. Assay Diluent A (Item D): 30 ml diluent buffer, 0.09% sodium azide as preservative. For Standard/Sample (serum/plasma) diluent.
5. Assay Diluent B (Item E): 15 ml of 5x concentrated buffer. For Standard/Sample (cell culture medium/urine) diluent.
6. Detection Affinity Reagent for PDGF-BB (Item F): 2 vials of a 4x concentrated solution of anti-human PDGF-BB affinity reagent.
7. Primer Solution (Item G): 1.7mL vial
8. PCR Master Mix (Item H): 1.2mL vial

9. PCR Preparation buffer (Item I): 1mL vial of 10x concentrated buffer
10. Final Wash Buffer (Item J): 10ml vial of 10x concentrated buffer

III. STORAGE

May be stored for up to 6 months at 2º to 8ºC from the date of shipment. Standard (recombinant protein) should be stored at -20 ºC or -80 ºC (recommended at –80 ºC) after reconstitution. Opened PCR plate or 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.
4 100 ml and 1 liter graduated cylinders.
5 Absorbent paper.
6 Distilled or deionized water.
7 Log-log graph paper or computer and software for data analysis.
8 Tubes to prepare standard or sample dilutions.
9 Heating block or water bath capable of 80^{O}C

V. REAGENT PREPARATION

1. Bring wash buffer, samples, assay diluents, and PCR plate to room temperature (18 - 25°C) before use. PCR master mix (Item H) and Primer solution (Item G) should be kept at 4^{O}C at all times.

2. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) should be used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) should be used for dilution of culture supernatants and urine.

Suggested dilution for normal serum/plasma: 3-50 fold*.

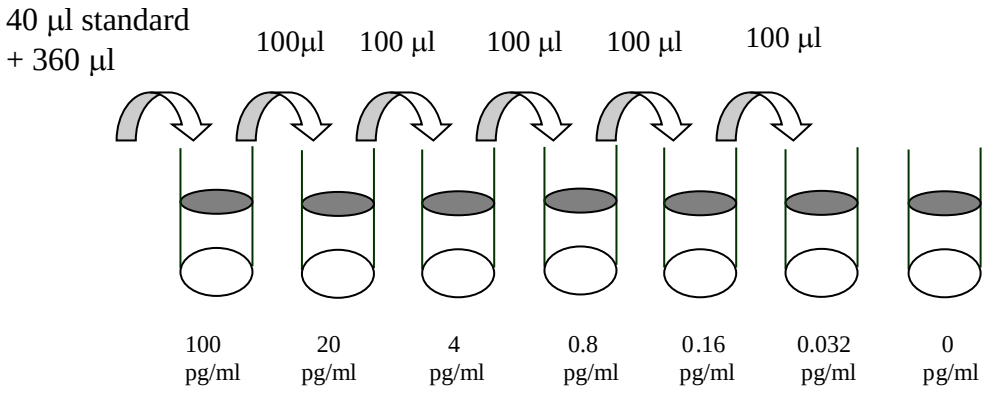
* Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

3. Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.

4. PCR preparation buffer (Item I) should be transferred to a 15mL tube and diluted with 9mL of deionized or distilled water before use.

5. Final Wash Buffer (Item J) 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.

6. Preparation of standard: **Briefly spin the vial of Item C** and then add 400 µl Assay Diluent A (Item D) to the vial to prepare a 1 ng/ml standard. **Dissolve the powder thoroughly by gentle mixing.** Add 40 µl PDGF-ββ standard from the vial of Item C, into a tube with 360 µl Assay Diluent A to prepare a 100 pg/ml standard solution. Use the 100 pg/ml standard solution to produce a dilution series (shown below) by pipetting 400µl of Assay Dilutent A into each tube and transferring 100µL for the previous standard into the new tube. Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/ml).



7. If the Wash Buffer Concentrate (10x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.

8. For preparation of detection affinity reagent (Item F) should be heated to 80^{O}C for 10 minutes before being placed immediately on ice for 15 minutes. After incubation transfer to a 15mL tube and mix with 3mL of 1x assay diluent B (Item E).

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. The plate may be broken into strips so that you only use the amount you need, and the rest can be saved for use at a later date.

2. Add 10-25µl of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Volumes should be consistent between all wells, samples, and standards. As little as 10µL can be used if sample volume is limited, however this increases the chance of technical error. Ensure there are no bubbles present at the bottom of the wells. Dislodge any bubbles with gentle tapping or with a pipette tip being careful not to contact the sides or bottom of the well. Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.

3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (100 µl) 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.

4. Add 25 µl of prepared detection affinity reagent (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution. Repeat the wash as in step 3.

6. Add 100µL of Final wash buffer (Item J) to each well and incubate for 5 minutes with rocking. Remove the solution from each well and repeat an additional 2x.

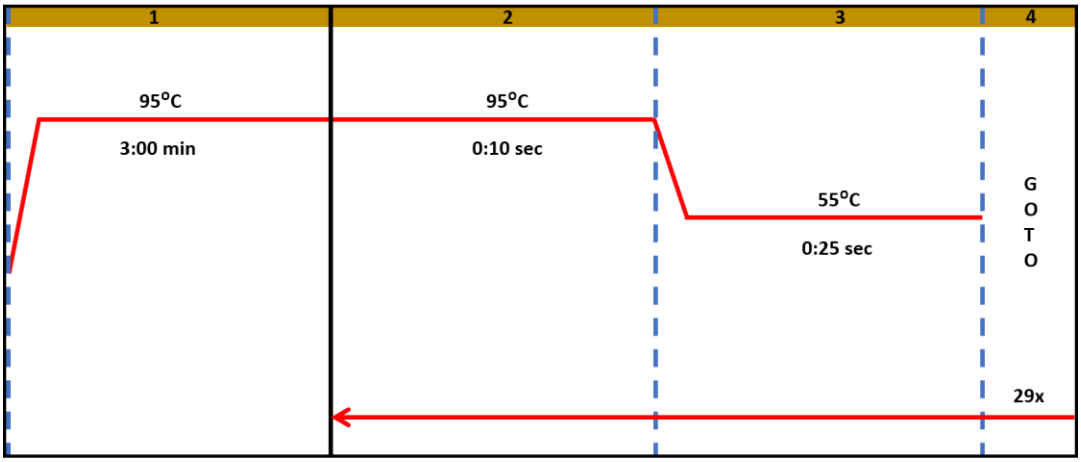
7. Add 100µL of 1x PCR preparation buffer (Item I) to each well and incubate with rocking for 5 minutes before removing the buffer. Blot the plate after the buffer is removed to ensure complete removal of the buffer.

8. Add 15µL of the Primer solution (Item G) to each well of the plate. At this stage the plate can be covered and stored at -20^{O}C for use the next day if needed.

9. Add 10µL of PCR Master Mix (Item H) to each well and pipette thoroughly to mix the well (at least 3x up and down).

10. Cover the plate with the supplied caps taking care to press firmly on each well, ensuring that tubes are completely closed. It may be helpful to remove each strip from the others and cap individually.

11. Place the plate into a real-time PCR instrument using a FITC 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, samples and standards as instructed.

2. Add 25µl standard or sample to each well.
   Incubate 2.5 hours at room temperature or overnight at 4ºC.

3. Add 25µl detection affinity reagent to each well.
   Incubate 1 hour at room temperature.

4. Add 15µl Primer solution to each well

5. Add 10µL of PCR master mix to each well

6. Run real-time PCR

VIII. CALCULATION OF RESULTS

The primary data output of the IQELISA kit is C_t 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_t for each set of triplicate standards, controls and samples. Subtract the C_t 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_t is a log based data.

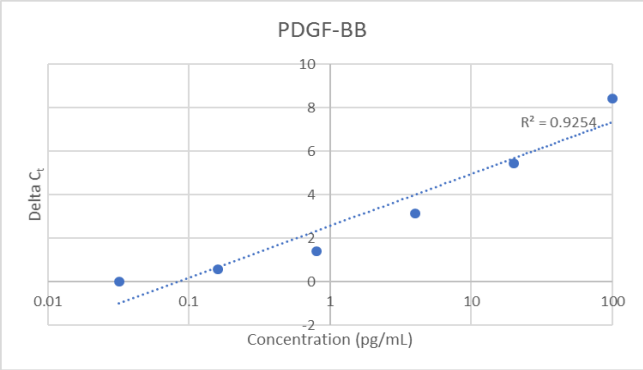
The line of best fit will have an equation y = mln(x)+b, where y is the Delta C_t value and x is the concentration. It may be helpful to use 5 significant figures for m and b to minimize rounding errors. To calculate the concentration of unknown sample this can be entered into excel in the following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

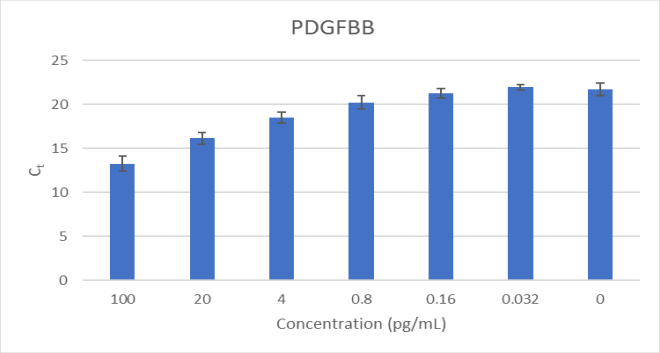
=EXP((y-b)/m))

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

A. TYPICAL DATA

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





B. SENSITIVITY and RECOVERY

The minimum quantifiable dose of PDGF-ββ is typically 0.2 pg/ml.

Serum spike tests show recovery is 93.1% with a range of 90.4 to 97.1%

Intraplate CV is below 10% for all samples and Interplate CV is below 15%

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. 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 IQELISA kit	1. Store your standard at<-20°C after reconstitution, others at 4 °C.
	2. Improper Tm	2. Check run parameters and calibrate instrument

This product is for research use only.