RayBio® Human GATA1/Eryf1 ELISA Kit

Catalog #: ELH-GATA1

User Manual
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Caution:
Extraordinarily useful information enclosed

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RayBio® Human GATA1/Eryf1 ELISA Kit Protocol

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Please read the entire manual carefully before starting your experiment

I. INTRODUCTION

The RayBio® Human GATA1/Eryf1 ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human GATA1/Eryf1 in serum, plasma and cell culture supernatants. This assay employs an antibody specific for human GATA1/Eryf1 coated on a 96-well plate. Standards and samples are pipetted into the wells and GATA1/Eryf1 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human GATA1/Eryf1 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of GATA1/Eryf1 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

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II. STORAGE

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see table below.

III. REAGENTS

Component	**Size / Description**	**Storage / Stability After Preparation**
GATA1/Eryf1 Microplate (Item A)	96 wells (12 strips x 8 wells) coated with anti-Human GATA1/Eryf1.	1 month at 4°C*
Wash Buffer Concentrate (20X) (Item B)	25 ml of 20X concentrated solution.	1 month at 4°C
Standard Protein (Item C)	2 vials of Human GATA1/Eryf1. 1 vial is enough to run each standard in duplicate.	1 week at -80°C
Detection Antibody GATA1/Eryf1 (Item F)	2 vials of biotinylated anti-Human GATA1/Eryf1. Each vial is enough to assay half the microplate.	5 days at 4°C
HRP-Streptavidin Concentrate (Item G)	200 µl 250X concentrated HRP-conjugated streptavidin.	Do not store and reuse.
TMB One-Step Substrate Reagent (Item H)	12 ml of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution.	N/A
Stop Solution (Item I)	8 ml of 0.2 M sulfuric acid.	N/A
Assay Diluent D (Item K)	15 ml of 5X concentrated buffer.	1 month at 4°C
Assay Diluent B (Item E)	15 ml of 5X concentrated buffer.	1 month at 4°C

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

IV. ADDITIONAL MATERIALS REQUIRED

1. Microplate reader capable of measuring absorbance at 450 nm.
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 ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

V. REAGENT PREPARATION

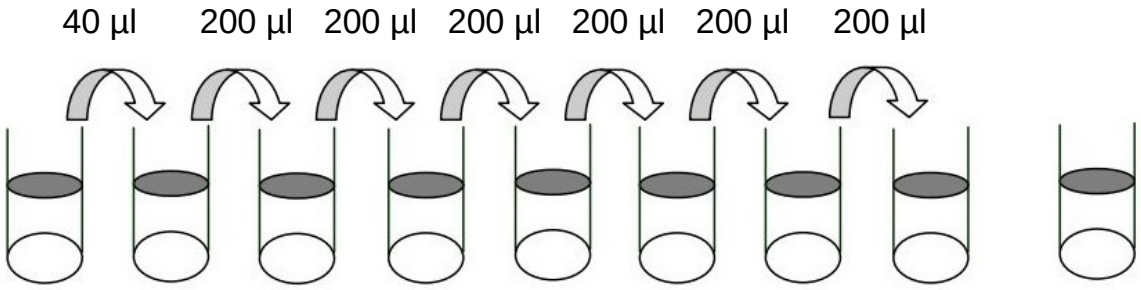
1. Bring all reagents and samples to room temperature (18 - 25ºC) before use.

2. Assay Diluent D (Item K) and Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water before use.

3. Sample dilution: 1X Assay Diluent D (Item K) should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2 fold.

   Note: Levels of GATA1/Eryf1 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

4. Preparation of standard: Briefly spin a vial of Item C. Add 400 µl 1X Assay Diluent D (Item K, Assay Diluent D should be diluted 5-fold with deionized or distilled water before use) into Item C vial to prepare a 50 ng/ml standard solution. Dissolve the powder thoroughly by a gentle mix. Add 40 µl of GATA1/Eryf1 standard solution from the vial of Item C, into a tube with 460 µl 1X Assay Diluent D to prepare a 4000 pg/ml standard solution. Pipette 300 µl 1X Assay Diluent D into each tube. Use the 4000 pg/ml standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1X Assay Diluent D serves as the zero standard (0 pg/ml).



		Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Item C + 400 µl	460 µl	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl		300 µl
Conc.	50 ng/ml	4000 pg/ml	1600 pg/ml	640 pg/ml	256 pg/ml	102.4 pg/ml	40.96 pg/ml	16.38 pg/ml		0 pg/ml

5. If the Wash Concentrate (20X) (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.

6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1X Assay Diluent B (Item E) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4ºC for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B (Item E) and used in step 5 of Part VI Assay Procedure.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 250-fold with 1X Assay Diluent B (Item E).

   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 40 µl of HRP-Streptavidin concentrate into a tube with 10 ml 1x Assay Diluent B to prepare a 250-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

VI. ASSAY PROCEDURE

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use. It is recommended that all standards and samples be run at least in duplicate.

2. Label removable 8-well strips as appropriate for your experiment.

3. Add 100 µl of each standard (see Reagent Preparation step 3) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.

4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 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.

5. Add 100 µl of 1X prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

6. Discard the solution. Repeat the wash as in step 4.

7. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

8. Discard the solution. Repeat the wash as in step 4.

9. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

10. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

VII. ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards as instructed.

2. Add 100 µl standard or sample to each well. Incubate 2.5 hours at room temperature.

3. Add 100 µl prepared biotin antibody to each well. Incubate 1 hour at room temperature.

4. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at room temperature.

5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

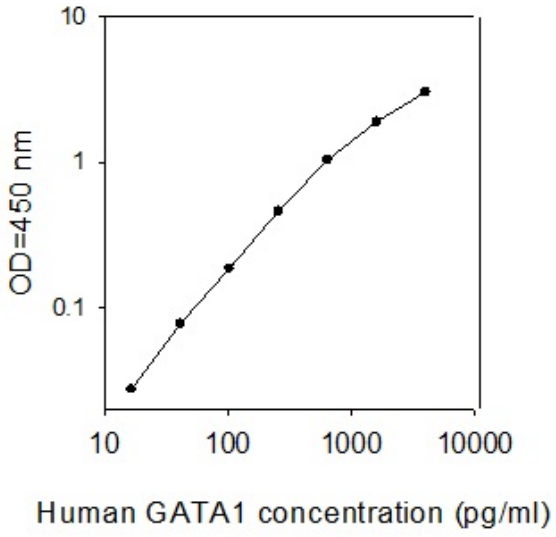
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

VIII. CALCULATION OF RESULTS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

A. TYPICAL DATA

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



B. SENSITIVITY

The minimum detectable dose of Human GATA1/Eryf1 was determined to be 16 pg/ml.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

C. SPIKING & RECOVERY

Recovery was determined by spiking various levels of Human GATA1/Eryf1 into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	94.15	70-133
Plasma	71.13	67-79
Cell culture media	96.31	86-106

D. LINEARITY

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	116.3	81.31	92.31
	Range (%)	113-120	71-95	89-95
1:4	Average % of Expected	100.5	80.92	77.41
	Range (%)	98-103	72-98	70-84

E. REPRODUCIBILITY

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

IX. SPECIFICITY

This ELISA antibody pair detects human GATA1. Other species not determined.

X. TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	• Inaccurate pipetting • Improper standard dilution	• Check pipettes • Briefly centrifuge Item C and dissolve the powder thoroughly by gently mixing
Low signal	• Improper preparation of standard and/or biotinylated antibody • Too brief incubation times • Inadequate reagent volumes or improper dilution	• Briefly spin down vials before opening. Dissolve the powder thoroughly. • Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4°C with gentle shaking (note: may increase overall signals including background). • Check pipettes and ensure correct preparation
Large CV	• Inaccurate pipetting • Air bubbles in wells	• Check pipettes • Remove bubbles in wells
High background	• Plate is insufficiently washed • Contaminated wash buffer	• Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed. • Make fresh wash buffer
Low sensitivity	• Improper storage of the ELISA kit • Stop solution	• Store your standard at <-70ºC after reconstitution, others at 4ºC. Keep substrate solution protected from light. • Add stop solution to each well before reading plate

RayBio® ELISA Kits

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