Rabbit GDF5 ELISA Kit

ARG83212 Rabbit GDF5 ELISA Kit is an Enzyme Immunoassay kit for the quantification of Rabbit GDF5 in serum, plasma (heparin, EDTA) and cell culture supernatants

Catalog number: ARG83212

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION **Page**

INTRODUCTION 3
PRINCIPLE OF THE ASSAY 3
MATERIALS PROVIDED & STORAGE INFORMATION 4
MATERIALS REQUIRED BUT NOT PROVIDED 4
TECHNICAL HINTS AND PRECAUTIONS 5
SAMPLE COLLECTION & STORAGE INFORMATION 6
REAGENT PREPARATION 7
ASSAY PROCEDURE 10
CALCULATION OF RESULTS 12
EXAMPLE OF TYPICAL STANDARD CURVE 13
QUALITY ASSURANCE 13

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Phone: +886 (3) 622 1320

Fax: +886 (3) 553 0266

Email: info@arigobio.com

INTRODUCTION

The protein encoded by this gene is a member of the bone morphogenetic protein (BMP) family and the TGF-beta superfamily. This group of proteins is characterized by a polybasic proteolytic processing site which is cleaved to produce a mature protein containing seven conserved cysteine residues. The members of this family are regulators of cell growth and differentiation in both embryonic and adult tissues. Mutations in this gene are associated with acromesomelic dysplasia, Hunter-Thompson type; brachydactyly, type C; and chondrodysplasia, Grebe type. These associations confirm that the gene product plays a role in skeletal development. [provided by RefSeq, Jul 2008]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for GDF5 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any GDF5 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for GDF5 is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of GDF5 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of GDF5

in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C or at-20°C for long-term storage. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C
Standard (Lyophilized)	2 X 10 ng/vials	4°C
Standard/Sample diluent	30 ml (Ready to use)	4°C
Antibody conjugate concentrate (100X)	1 vial (100 µl)	4°C
Antibody diluent buffer	12 ml (Ready to use)	4°C
HRP-Streptavidin concentrate (100X)	1 vial (100 µl)	4°C
HRP-Streptavidin diluent buffer	12 ml (Ready to use)	4°C
25X Wash Buffer	20 ml	4°C
TMB substrate	10 ml (Ready to use)	4°C (Protect from light)
STOP solution	10 ml (Ready to use)	4°C
Plate sealer	4 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water

- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times. The kit can also be stored at-20°C for long-term storage.
- To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
- The TMB Color developing agent should be colorless and transparent before using.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- If crystals are observed in the 25X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Do not let strips dry, as this will inactivate active components in wells.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Avoid using reagents from different batches.

- In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the 1X HRP-Streptavidin Solution and TMB substrate be pre-warmed in 37°C for few minutes before use.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at -20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.

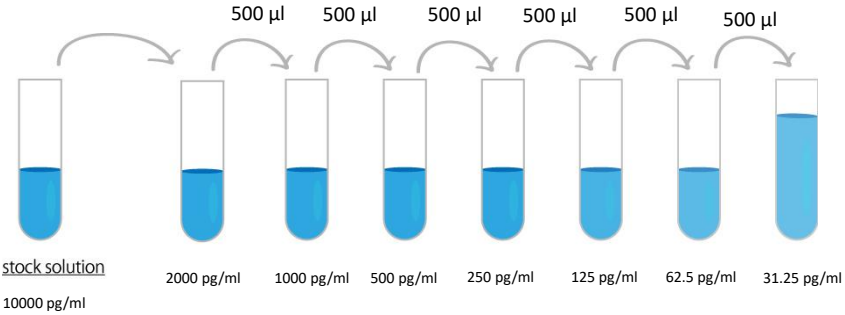
b) Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 25 X wash buffer with deionized water to yield 1X wash buffer. The pH value of dissolved 1X wash buffer should between pH7.2 to pH7.6. The diluted 1X wash buffer is stable for a week at 2°C to 8°C.
- 1X Antibody conjugate: It is recommended to prepare this reagent immediately prior to use and use it within 2 hours after preparation. Dilute 100X antibody conjugate concentrate into Antibody diluent buffer to yield 1X detection antibody solution. (e.g. 10 µl of 100X antibody conjugate concentrate + 990 µl of Antibody diluent buffer)
- 1X HRP-Streptavidin Solution: It is recommended to prepare this reagent immediately prior to use and use it within 1 hours after preparation. Dilute 100X HRP-Streptavidin concentrate solution into HRP-Streptavidin diluent buffer to yield 1X HRP-Streptavidin Solution buffer. (e.g. 10 µl of 100X HRP-Streptavidin concentrate solution + 990 µl of HRP-Streptavidin diluent buffer)
- Sample: If the initial assay found samples contain GDF5 higher than the highest standard, the samples can be diluted with Standard/Sample diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with the diluent buffer before assay. Serum sample can be diluted 2X with Standard/Sample diluent before assay. (One part of serum sample add into one part of Standard/Sample diluent.)
  (It is recommended to do pre-test to determine the suitable dilution factor).

- **Standards**: Standard solution should be prepared within 2 hours prior to the experiment. Reconstitute the standard with **1 ml** of **Standard/Sample diluent** to yield a stock concentration of **10000 pg/ml**. Allow the stock standard to sit for at least 10 minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The Standard/ Sample diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Standard/ Sample diluent as according to the suggested concentration below: 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml.

  Note: The reconstituted standard solutions are best used within 2 hours. The stock standard solution should be stored at 4°C for up to 12 hours, or aliquot & store at-20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.



Dilute GDF5 standard as according to the table below:

Standard	GDF5 Conc. (pg/ml)	µl of Standard/Sample diluent	µl of standard
S7	2000 pg/ml	800	200(10000 pg/ml Stock)
S6	1000 pg/ml	500	500 (S7)
S5	500 pg/ml	500	500 (S6)
S4	250 pg/ml	500	500 (S5)
S3	125 pg/ml	500	500 (S4)
S2	62.5 pg/ml	500	500 (S3)
S1	31.25 pg/ml	500	500 (S2)
S0	0	500	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) or 37°C before use. The 1X HRP-Streptavidin Solution and TMB substrate should be prewarm at 37°C few minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard GDF5 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of GDF5 amount in samples. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **standards, samples and zero control** (S0, Standard/Sample diluent) into wells. Cover the plate and incubate for **90 minutes at 37°C.**
3. **Aspirate each well**. Complete removal of liquid by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry at any time. Wash step is not necessary in this step.
4. Add **100 µl** of **1X Antibody conjugate** into each well, gently tap the plate to mix well. Cover wells and **incubate for 60 minutes at 37°C**.
5. Aspirate each well and wash, repeating the process two times for a total **three washes**. Wash by filling each well with **1X Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher, keep the wash buffer in the wells for 1 min before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry at any time.
6. Add **100 µl** of **1X HRP-Streptavidin solution** to each well, gently tap the

plate to mix well. Cover wells and **incubate for 30 minutes at 37°C**.

7. Aspirate each well and wash, repeating the process four times for a total **five washes**. Wash by filling each well with **1X Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher, keep the wash buffer in the wells for 1 min before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry at any time.
8. Add **90 µl** of **TMB substrate** to each well. Incubate for **15-25 minutes at 37°C** in dark. (Note: The incubation time is for reference only, the optimal incubation time should be determined by end user. And the shades of blue color can be seen in the wells with the four most concentrated GDF5 standard solutions; the other wells show no obvious color).
9. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing.
10. Read the OD with a microplate reader at **450 nm** immediately. It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, control and samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

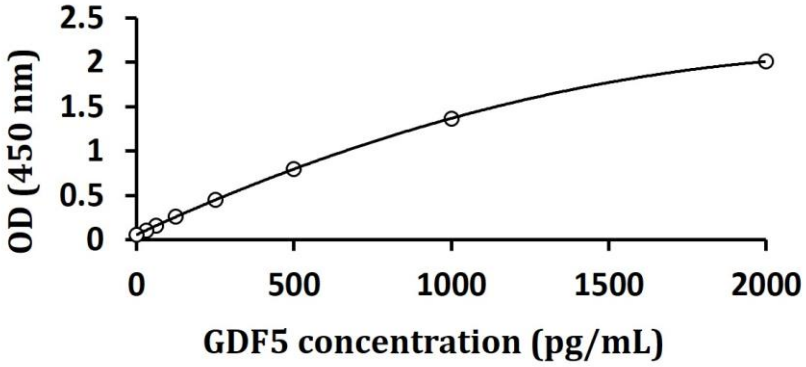
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Rabbit GDF5 ranged from 31.2 pg/ml - 2,000 pg/ml. The mean MDD was 15 pg/ml.

Specificity

This assay recognizes natural and recombinant Rabbit GDF5. There is no detectable cross-reactivity with other relevant proteins.

Intra-assay and Inter-assay precision

The CV values of intra-assay was < 10% and inter-assay was < 10%.