QUIDEL

MicroVue™ Bone
Human FGF-23 (C-Term) EIA

For the determination of human fibroblast growth factor 23 levels in plasma or cell culture media.

For **Research Use Only**. Not for use in diagnostic procedures.

A symbols glossary can be found at quidel.com/glossary.

INTRODUCTION

Fibroblast growth factor 23 (FGF-23) is a novel member of a large family of related proteins. The amino-terminal portion of FGF-23 (aa 1-24) is hydrophobic and is likely to serve as a signal peptide allowing its secretion into the blood circulation. Its carboxyl-terminal portion (aa 180-251) shares only limited amino acid homology with other members of the FGF family of proteins.

Renal phosphate wasting disorders leading to hypophosphatemia are among the causes of defective mineralization of bone and growth plate development. Patients with autosomal dominant hypophosphatemic rickets (ADHR), a rare genetic disorder, carry one of several different FGF-23 mutations that make the protein resistant to proteolytic cleavage. Furthermore, tumors that cause oncogenic osteomalacia (OOM) have been shown to overexpress FGF-23 mRNA making it likely that elevated concentrations of FGF-23 in the blood are the cause of renal phosphate wasting in this group of patients. Consistent with this conclusion, the administration of recombinant FGF-23 to rodents was shown to increase urinary excretion of phosphate thus leading to hypophosphatemia and osteomalacia/rickets. Taken together, all currently available data suggest that FGF-23 is either directly or indirectly involved in the regulation of phosphate homeostasis.

The measurement of human FGF-23 levels in the blood is likely to provide an important diagnostic tool for the laboratory evaluation of patients with a variety of different hypophosphatemic and hyperphosphatemic disorders. Furthermore, the sensitive measurement of FGF-23 is likely to provide novel insights into the regulation of bone and mineral homeostasis.

TEST PRINCIPLE

This 2nd generation Human FGF-23 (C-Term) ELISA Kit is a two-site enzyme-linked immunosorbent assay (ELISA) for the measurement of FGF-23 in plasma or cell culture media. Two affinity purified goat polyclonal antibodies have been selected to detect epitopes within the carboxyl-terminal (C-Term) portion of FGF-23. One antibody is biotinylated for capture and the other antibody is conjugated with the enzyme horseradish peroxidase (HRP) for detection. These antibodies bind to both the intact molecule and large carboxyl terminal fragments of human FGF-23.

A sample containing human FGF-23 is incubated simultaneously with the biotinylated capture antibody and the HRP conjugated antibody in a streptavidin coated microtiter well. FGF-23 contained in the sample is immunologically bound by the capture antibody and the detection antibody to form a “sandwich” complex:

Well/Avidin — Biotin Anti-h FGF23 (C-terminal) — Human FGF23 — HRP Anti-h FGF23 (C-terminal)

At the end of this incubation period, the well is washed to remove any unbound antibody and other components. The enzyme bound to the well is incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microtiter plate reader. The enzymatic activity of the antibody complex bound to the well is directly proportional to the amount of FGF-23 in the sample. A standard curve is generated by plotting the absorbance versus the respective FGF-23 concentration for each standard on linear or logarithmic scales. The concentration of human FGF-23 in the samples is determined directly from this curve.

REAGENTS AND MATERIALS SUPPLIED

MicroVue Human FGF-23 (C-Term) EIA Kit contains the following:

Srreptavidin Coated Microtiter Plate 40-0010 1 plate

Twelve (12) eight well strips (96 wells total). This reagent should be stored in the foil pouch with desiccant at 2°C to 8°C and is stable until the expiration date on the kit.

Biotinylated Human FGF-23 Antibody 40-6110 2.7 mL

One (1) vial of biotin labeled anti-human FGF-23 in TRIS buffered saline with protein stabilizers and 0.1% Ciprofloxacin as preservative. This reagent should be stored at 2°C to 8°C and is stable until the expiration date on the kit.

HRP Conjugated Human FGF-23 Antibody 40-6120 2.7 mL

One (1) vial of horseradish peroxidase (HRP) conjugated to anti-human FGF-23 in a stabilized protein solution with 0.1% Ciprofloxacin as preservative. This reagent should be stored at 2°C to 8°C protected from light and is stable until the expiration date on the kit.

NOTE: Make a working Antibody Solution by pipetting equal volumes of Biotinylated Human FGF-23 Antibody and HRP Conjugated Human FGF-23 Antibody prior to use. Mix only the volume required for immediate use. Mix well to ensure homogeneity.

Human FGF-23 Standards 40-6131 to 40-6136

Six (6) vials each containing recombinant human FGF-23 lyophilized in a protein matrix with 0.1% Ciprofloxacin as preservative. **Refer to vial label for exact concentration.** Before use reconstitute the vial with the FGF-23 concentration of 0 RU/mL with 2.0 mL of deionized water. Before use reconstitute each of the other five vials of standards with 1.0 mL of deionized water. Allow the vials to sit for approximately 20 minutes with occasional gentle swirling and inversion. Assure complete reconstitution before use.

Use the standards immediately after reconstitution; freeze the unused portion for later use. After reconstitution the standards are stable until the expiration date on the kit when stored at –20°C or below with up to 3 freeze/thaw cycles.

Human FGF-23 Controls I & II 40-6141 and 40-6142

Two (2) vials each containing recombinant human FGF-23 lyophilized in a protein matrix with 0.1% Ciprofloxacin as preservative. **Refer to vial label for control ranges.** Before use reconstitute each control with 1.0 mL of deionized water. Allow the vials to sit for approximately 20 minutes with occasional gentle swirling and inversion. Assure complete reconstitution before use.

Use the controls immediately after reconstitution; freeze the unused portion for later use. After reconstitution the controls are stable until the expiration date on the kit when stored at –20ºC or below with up to 3 freeze/thaw cycles.

ELISA Wash Concentrate **(40-0041)** **20 mL**

One (1) vial of a 20-fold concentrate. Before use dilute the contents to 400 mL with deionized water and mix well. Upon dilution this yields a working wash solution containing a surfactant in phosphate buffered saline with 0.1% Ciprofloxacin as preservative. The diluted wash solution should be stored at room temperature and is stable until the expiration date on the kit.

ELISA HRP SUBSTRATE **40-0027** **16 mL**

One (1) bottle of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2°C to 8°C protected from light and is stable until the expiration date on the kit.

ELISA Stop Solution **40-0030** **11 mL**

One (1) bottle of 1 M sulfuric acid. This reagent may be stored at room temperature or at 2°C to 8°C and is stable until the expiration date on the kit.

Plate Sealer **10-2016**

Two (2) included in kit.

Human FGF-23 Sample Diluent (Optional reagent, must be ordered separately using Cat. #30-6631) **10 mL**

One (1) bottle of a lyophilized protein matrix with 0.1% Ciprofloxacin as preservative. This reagent should be stored at 2°C to 8°C and is stable until the expiration date on the bottle. Before use reconstitute with 10 mL of deionized water. Allow the bottle to sit for approximately 20 minutes with occasional gentle swirling and inversion. Assure complete reconstitution before use. Aliquot and freeze (–20°C or below) any unused portion for later use.

MATERIALS REQUIRED BUT SUPPLIED IN KIT

- 1.0 mL and 2.0 mL volumetric pipettes for reconstituting standards and controls.
- Precision pipets capable of delivering 50 µL ,100 µL and 150 µL.
- Aluminum foil.
- Automated microtiter plate washer OR
- Repeating dispenser for delivering 350 µL and suitable aspiration device.
- Container for storage of wash solution.
- Spectrophotometric microtiter plate reader capable of reading absorbance at 450 nm and at 595-650 nm.
- Deionized water.
- Horizontal rotator capable of maintaining 180-220 RPM.
- Timer

WARNINGS AND PRECAUTIONS

- Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid (i.e. ELISA HRP Substrate and ELISA Stop Solution). In case of contact with any of these reagents, wash thoroughly with water. TMB is a suspected carcinogen. Use Good Laboratory Practices. Wash hands before eating. Do not eat, drink or smoke in the work area.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

Preparation and Storage

Prior to use allow all reagents to come to room temperature and mix by gentle swirling and inversion. Reagents from different kit lot numbers should not be combined or interchanged.

Store the kit at 2°C to 8°C upon receipt. **Store the standards and controls at –20°C or below after reconstitution.** For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

SPECIMEN COLLECTION

The FGF-23 molecule appears to be unstable resulting in decreased immunoreactivity over time. Sample collection and storage procedures should be carried out in an expeditious manner. **Due to the variable lability of the molecule, measurement of the FGF-23 concentration should be made using EDTA plasma or cell culture media.** Two hundred microliters of plasma or culture media are required to assay the sample in duplicate. Centrifuge the sample and separate the plasma or media from the cells. Samples should be assayed immediately or stored frozen at –20°C or below. Avoid repeated freezing and thawing of specimens.

ASSAY PROCEDURE

1. Place a sufficient number of Streptavidin Coated Strips in a holder to run FGF-23 standards, controls and samples.
2. Pipet 100 µL of standard, control, or sample into the designated or mapped well. Freeze the remaining standards and controls as soon as possible after use.
3. Pipet 50 µL of the Working Antibody Solution consisting of 1 part Biotinylated Antibody and 1 part HRP Antibody into each well.
4. Cover the plate with one plate sealer, then cover with aluminum foil to avoid exposure to light.
5. Incubate plate at room temperature for three (3) hours on a horizontal rotator set at 180-220 RPM.
6. Remove the aluminum foil and plate sealer. **Using an automated microtiter plate washer aspirate the contents of each well. Wash each well five times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents.** A suitable aspiration device may also be used.
7. Pipet 150 µL of ELISA HRP Substrate into each of the wells.
8. Re-cover the plate with a plate sealer and aluminum foil. Incubate at room temperature for 30 minutes on a horizontal rotator set at 180-220 RPM.
9. Remove the aluminum foil and plate sealer. Read the absorbance at 620 nm (see Note) within 5 minutes in a microtiter plate reader against the 0 RU/mL Standard wells as a blank.
10. Immediately pipet 50 µL of ELISA Stop Solution into each of the wells. Mix on horizontal rotator for 1 minute.
11. Read the absorbance at 450 nm within 10 minutes in a microtiter plate reader against a reagent blank of 150 µL of Substrate and 50 µL of Stop Solution.

    If dual wavelength correction is available, set the Measurement wavelength to 450 nm and Reference wavelength to absorbance used in step #9.

    NOTE: Absorbance may be read at wavelengths from 595 nm to 650 nm depending upon available filters.

PROCEDURAL NOTES

- It is recommended that all standards, controls and samples be assayed in duplicate. The average absorbance reading of each duplicate should then be used for data reduction and the calculation of results.
- Keep light sensitive reagents (i.e. HRP Conjugated Antibody, the Working Antibody Solution consisting of combined Biotinylated Antibody and HRP Conjugated Antibody, and ELISA HRP Substrate) in the original amber bottles or other suitable container which is well protected from light.

- Store any unused Streptavidin Coated Strips in the resealable aluminum pouch with desiccant to protect from moisture.
- The sample and all reagents should be pipetted carefully to minimize air bubbles in the wells.
- The sequence and timing of each reagent addition is important as both the immunological and enzymatic reactions are in kinetic modes. The washing step is also an important part of the total assay procedure. **The use of an automated microtiter plate washer is strongly recommended.** All pipetting and washing steps should be performed such that the timing is as consistent as possible.
- Samples with values greater than the highest standard should be diluted 1:10 or greater with the 0 RU/mL Standard or optional Sample Diluent reagent and reassayed. Multiply the result by the dilution factor. (See Limitations, # 2)
- Plasma or cell culture media samples may contain fibrin clots or cellular debris. Freeze/thaw of plasma samples may accelerate clot formation. These samples must be centrifuged and decanted prior to assay to remove all particulate material which can cause random high non-specific binding on well surface.
- Rarely, upon opening the streptavidin plate, small white crystals may be observed in some of the wells. This is entirely cosmetic and will not affect the assay. This condition is reported by other kit manufacturers and results from the final stabilizing buffer used in the coating process.

CALCULATION OF RESULTS

The two absorbance readings taken before and after the addition of the ELISA Stop Solution allow for the construction of two standard curves using the human FGF-23 standards contained in the kit. **Refer to the individual vial label for exact concentration.** The primary curve used for calculation of results is the second reading taken after the addition of the ELISA Stop Solution and read at 450 nm. This data utilizes the absorbance values obtained with the first five standards. The first reading taken before the addition of the ELISA Stop Solution and read at 595 nm-650 nm is intended to extend the analytical range to the value of the sixth (highest) standard provided in the kit. **It should be used only for sample results that fall between the value of the fifth and sixth standard.** Results obtained with this reading should not replace the on-scale reading at 450 nm. Each curve should be generated as follows:

Primary Procedure—Read at 450 nm

1. Calculate the average absorbance for each pair of duplicate assay wells.
2. Subtract the average absorbance of the 0 RU/mL Standard from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by plotting the corrected absorbance of the first five standard levels on the ordinate against the standard concentration on the abscissa using linear-linear or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.

The FGF-23 concentration of the controls and samples are read directly from the standard curve using their respective corrected absorbance. If log-log graph paper or computer assisted data reduction programs utilizing logarithmic transformation are used, samples having corrected absorbance between the 0 RU/mL Standard and the next highest standard should be calculated by the formula:

$$\text{Value of unknown} = \frac{\text{Corrected Absorbance (unknown)}}{\text{Corrected Absorbance (2nd Std.)}} \times \text{Value of the 2nd Std.}$$

Secondary Procedure—Read at 595 nm-650 nm

1. Calculate the average absorbance for each pair of duplicate assay wells.
2. The standard curve is generated by plotting the absorbance of the three highest standards on the ordinate against the standard concentration on the abscissa using linear-linear or log-log graph paper.

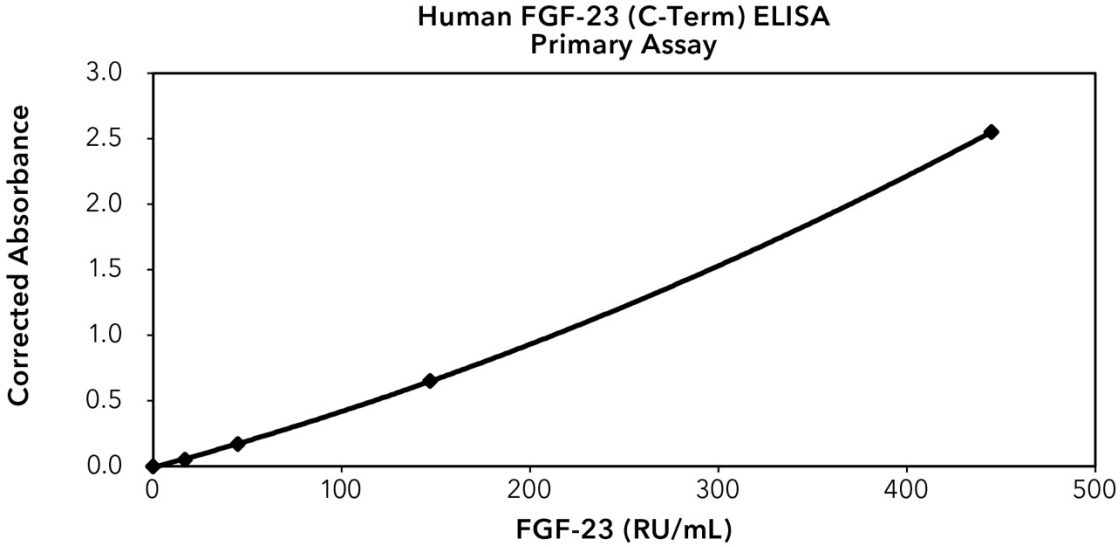
3. The FGF-23 concentration of samples reading only between the fifth and sixth standard are read directly from this standard curve.

EXAMPLE DATA AND STANDARD CURVE

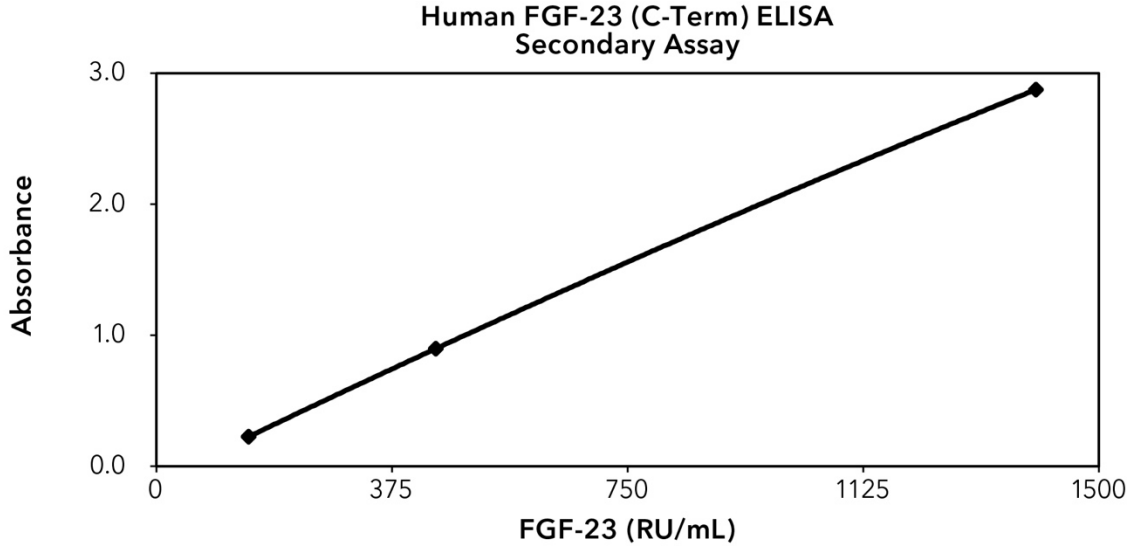
The following are representative examples of data and the resulting standard curves from the primary and secondary procedures. **These curves should not be used in lieu of a standard curve run with each assay.**

Primary Assay—450 nm				
Well I.D.	**ABS**	**Average ABS**	**Corrected ABS**	**Results RU/mL**
Reagent Blank	0.000			
	0.000	0.000		
0 RU/mL	0.009			
	0.009	0.009	0.000	
17 RU/mL	0.059			
	0.059	0.059	0.050	
45 RU/mL	0.183			
	0.179	0.181	0.172	
147 RU/mL	0.659			
	0.661	0.660	0.651	
445 RU/mL	2.562			
	2.561	2.561	2.552	
Control I	0.113			
	0.117	0.115	0.106	30
Control II	1.577			
	1.552	1.564	1.555	289
Sample 1	0.310			
	0.319	0.315	0.306	73
Sample 2	2.745			
	2.768	2.756	2.747	*

* > 445 RU/mL. Calculate using secondary assay



Secondary Assay—620 nm			
Well I.D.	ABS	Average ABS	Results RU/mL
0 RU/mL	0.000		
	0.000	0.000	
147 RU/mL	0.230		
	0.223	0.227	
445 RU/mL	0.897		
	0.901	0.899	
1400 RU/mL	2.888		
	2.864	2.876	
Sample 2	0.960		
	0.969	0.965	477



LIMITATIONS OF THE PROCEDURE

- The lowest concentration of human FGF-23 measurable is 1.5 RU/mL (assay sensitivity) and the highest concentration of human FGF-23 measurable without dilution is the value of the highest standard.
- The reagents in this 2nd generation Human FGF-23 (C-Term) ELISA kit have been optimized so that the high dose “hook effect” is not a problem for samples with elevated FGF-23 values. Samples with levels between the highest standard and 750,000 RU/mL will read greater than the highest standard and should be diluted 1:10 or greater with the 0 RU/mL Standard or optional Sample Diluent reagent and reassayed for correct values.
- Grossly lipemic samples may affect the immunological response and it is recommended that results obtained with such samples be scrutinized accordingly.
- Differences in protein concentration and protein type between samples and standards in an immunoassay contribute to "protein effects" and dose biases. When measuring low protein concentration culture media samples against high protein concentration standards, it is recommended that like samples be assayed together in the same assay to minimize this bias.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known levels of human FGF-23. MicroVue recommends that all assays include the laboratory’s own human FGF-23 controls in addition to those provided with this kit.

PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of the 2nd generation Human FGF-23 (C-Term) ELISA as determined by the 95% confidence limit on 20 duplicate determinations of the 0 RU/mL Standard is 1.5 RU/mL.

Precision

To assess intra-assay precision the mean and coefficient of variation were calculated from 20 duplicate determinations of two samples each performed in a single assay.

Mean Value (RU/mL)	Coefficient of Variation
33.7	2.4 %
302	1.4 %

To assess inter-assay precision the mean and coefficient of variation were calculated from duplicate determinations of two samples performed in 10 assays.

Mean Value (RU/mL)	Coefficient of Variation
33.6	4.7 %
293	2.4 %

Parallelism

Human plasma samples were diluted with 0 RU/mL Standard and assayed. Results in RU/mL are as follows:

Sample	Dilution	Observed Value	Expected Value	% O/E
1	undiluted	60.0		
	1:2	27.9	30.0	93
	1:4	14.3	15.0	95
	1:8	6.8	7.5	91
2	undiluted	182		
	1:2	87.3	91.0	96
	1:4	51.5	45.5	113
	1:8	18.5	22.8	81
3	undiluted	312		
	1:2	116	156	74
	1:4	81.9	78	105
	1:8	44.5	39	114

Recovery

Various amounts of FGF-23 were added to three different human plasma samples and assayed. Results in RU/mL are as follows:

Sample	Orig. Value	Amount Added	Observed Value	Expected Value	% O/E
1	84	375	470	438	107
		750	891	792	112
		1125	1255	1146	110
2	79	375	394	434	91
		750	770	789	98

Sample	Orig. Value	Amount Added	Observed Value	Expected Value	% O/E
		1125	1148	1145	100
3	60	375	484	420	115
		750	906	780	116
		1125	1261	1140	111

ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at (740) 592-9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S.A., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website **quidel.com**.

REFERENCES

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60-6100 MicroVue Human FGF-23 (C-Term) EIA – 96 Test

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PI6061001EN00 (10/19)

GLOSSARY

REF Catalogue number	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Consult e-labeling instructions for use
RUO For Research use only	 Contains sufficient for 96 determinations
CONT Contents/Contains	CONTROL Control