



# **Rat/Mouse Fibroblast Growth Factor-21 (FGF-21)**

**96-Well Plate**

**Cat. # EZRMFGF21-26K**

# RAT/MOUSE FIBROBLAST GROWTH FACTOR-21 (FGF-21)

## ELISA KIT

### 96-Well Plate

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## I. INTENDED USE

This Rat/Mouse FGF-21 ELISA kit is used for the non-radioactive quantification of Rat/Mouse FGF-21 in serum, plasma, and adipocyte extracts or cell culture media samples. This kit specifically measures native Rat/Mouse FGF-21. One kit is sufficient to measure 39 unknown samples in duplicate. ***This kit is for Research Use Only. Not for Use in Diagnostic Procedures.***

## II. PRINCIPLES OF PROCEDURE

This assay is a Sandwich ELISA based, sequentially, on: 1) concurrent capture of Rat/Mouse FGF-21 molecules from samples to the wells of a microtiter plate coated with a polyclonal goat anti-FGF-21 antibody, and binding of a second biotinylated polyclonal goat anti-FGF-21 antibody to the captured molecules, 2) washing of unbound materials from samples, 3) binding of streptavidin-horseradish peroxidase conjugate to the immobilized biotinylated antibodies, 4) washing of excess of free enzyme conjugates, and 5) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetramethylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbance at 450 nm – 590nm after acidification of formed products. Since the increase in absorbance is directly proportional to the amount of captured Rat/Mouse FGF-21 in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of Rat/Mouse FGF-21.

### III. REAGENTS SUPPLIED

Each kit is sufficient to run one 96-well plate and contains the following reagents:

#### A. Rat/Mouse FGF-21 ELISA Plate

Coated with Goat anti-FGF-21 Antibodies

Quantity: 1 strip plate

Preparation: Ready to Use

Note: Unused strips should be resealed in the foil pouch with the desiccant provided.

#### B. Adhesive Plate Sealer

Quantity: 2 sheets

Preparation: Ready to Use

#### C. 10X HRP Wash Buffer Concentrate

10X concentrate of 50 mM Tris Buffered Saline containing Tween-20

Quantity: 2 bottles containing 50 mL each

Preparation: Dilute 1:10 with distilled or deionized water

#### D. Rat/Mouse FGF-21 Standard

Purified Recombinant Mouse FGF-21, lyophilized.

Quantity: 0.5 mL upon hydration

Preparation: Reconstitute with 0.5 mL distilled or deionized water.

See insert for concentration.

#### E. Rat/Mouse FGF-21 Quality Controls 1 and 2

One vial each, lyophilized, containing purified Recombinant Mouse FGF-21 at two different levels.

Quantity: 0.5mL/bottle upon hydration

Preparation: Reconstitute each vial with 0.5mL distilled or deionized water.

#### F. Matrix Solution

Quantity: 0.5mL

Preparation: Reconstitute with 0.5 mL distilled or deionized water. After reconstitution, dilute 1:2 by adding 0.5 mL Assay Buffer to the vial.

#### G. Assay Buffer

0.05M PBS, pH 6.8, containing proprietary protease inhibitors, with Tween 20, 0.08% Sodium Azide and 1% BSA.

Quantity: 12 mL

Preparation: Ready to Use

#### H. Rat/Mouse FGF-21 Detection Antibody

Pre-titered Biotinylated Goat anti-FGF-21 Antibody

Quantity: 1.2 mL

Preparation: Ready to Use

### **III. REAGENTS SUPPLIED (continued)**

#### **I. Enzyme Solution**

Pre-titered Streptavidin-Horseradish Peroxidase Conjugate in Buffer

Quantity: 12 mL

Preparation: Ready to Use

#### **J. Substrate (Light sensitive, avoid unnecessary exposure to light)**

3, 3', 5, 5'-tetramethylbenzidine in buffer

Quantity: 12 mL

Preparation: Ready to Use.

#### **K. Stop Solution (Caution: Corrosive Solution)**

0.3 M HCl

Quantity: 12 mL

Preparation: Ready to Use

### **IV. STORAGE AND STABILITY**

- Recommended storage for kit components is 2-8°C.
- All components are shipped and stored at 2-8°C. Reconstituted standards and controls can be frozen for future use but repeated freeze thaws should be avoided. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

### **V. REAGENT PRECAUTIONS**

#### **A. Sodium Azide**

Sodium Azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

#### **B. Hydrochloric Acid**

Hydrochloric Acid is corrosive and can cause eye and skin burns. It is harmful if swallowed and can cause respiratory and digestive tract burns. Avoid contact with skin and eyes. Do not swallow or ingest.

**Note: See Full Labels of Hazardous components on next page.**

**Full labels of hazardous components in this kit:**

Ingredient, Cat #		Full Label	
Rat/Mouse Fibroblast Growth Factor-21 Quality Controls 1 & 2	E6026-K		<b>Warning.</b> Harmful if swallowed. Toxic to aquatic life with long lasting effects. Avoid release to the environment.
Rat/Mouse Fibroblast Growth Factor-21 Standard	E8026-K		<b>Warning.</b> Harmful if swallowed. Toxic to aquatic life with long lasting effects. Avoid release to the environment.
Matrix Solution	EMTX-MSL	No Symbol Required	Harmful to aquatic life with long lasting effects. Avoid release to the environment.
Stop Solution	ET-TMB		<b>Warning.</b> May be corrosive to metals.
10X HRP Wash Buffer Concentrate	EWB-HRP		<b>Warning.</b> May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

## **VI. MATERIALS REQUIRED BUT NOT PROVIDED**

1. Pipettes and Pipette Tips: 10  $\mu$ L - 20  $\mu$ L or 20  $\mu$ L - 100  $\mu$ L
2. Multi-Channel Pipettes and Pipette Tips: 5  $\mu$ L ~ 50  $\mu$ L and 50  $\mu$ L ~ 300  $\mu$ L
3. Buffer and Reagent Reservoirs
4. Vortex Mixer
5. Deionized Water
6. Microtiter Plate Reader capable of reading absorbency at 450 nm
7. Orbital Microtiter Plate Shaker
8. Absorbent Paper or Cloth

## **VII. SAMPLE COLLECTION AND STORAGE**

1. To prepare serum samples, whole blood is directly drawn into a centrifuge tube that contains no anti-coagulant. Let blood clot at room temperature for 30 min.
2. Promptly centrifuge the clotted blood at 2,000 to 3,000xg for 15 minutes at  $4 \pm 2^{\circ}\text{C}$ .
3. Transfer and store serum samples in separate tubes. Date and identify each sample.
4. Use freshly prepared serum or aliquot and store samples at  $\leq -20^{\circ}\text{C}$  for later use. For long-term storage, keep at  $-70^{\circ}\text{C}$ . Avoid freeze/thaw cycles.
5. To prepare plasma samples, whole blood should be collected into centrifuge tubes containing enough  $\text{K}_3\text{EDTA}$  to achieve a final concentration of 1.735 mg/mL and centrifuged immediately after collection. Observe the same precautions in the preparation of serum samples.
6. If heparin is to be used as an anticoagulant, the effect on the assay outcome at the dose of heparin used should be pre-determined.
7. Avoid using samples with gross hemolysis or lipemia.

## VIII. REAGENT PREPARATION

### A. Rat/Mouse FGF-21 Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the Rat/Mouse FGF-21 Standard with 0.5 mL distilled or deionized water to give a concentration described on the analysis sheet. Invert and mix gently, let sit for 5 minutes then vortex gently.
2. Label five tubes 1, 2, 3, 4, and 5. Add 0.25 mL Assay Buffer to each of the five tubes. Prepare serial dilutions by adding 0.125 mL of the reconstituted standard to Tube 1, mix well and transfer 0.125 mL of Tube 1 to Tube 2, mix well and transfer 0.125 mL of Tube 2 to Tube 3, mix well and transfer 0.125 mL of Tube 3 to Tube 4, mix well and transfer 0.125 mL of Tube 4 to Tube 5 and mix well.

Note: Do not use a Repeater pipette. Change tip for every dilution. Wet tip with Standard before dispensing. Unused portions of reconstituted standard should be stored at  $\leq -20^{\circ}\text{C}$ . Avoid multiple freeze/thaw cycles.

Volume of Deionized Water to Add	Volume of Standard to Add	Standard Concentration (pg/mL)
0.5 mL	0	X (refer to analysis sheet for exact concentration)

Tube #	Volume of Assay Buffer to Add	Volume of Standard to Add	Standard Concentration (pg/mL)
Tube 1	0.25 mL	0.125 mL of reconstituted standard	X/3
Tube 2	0.25 mL	0.125 mL of Tube 1	X/9
Tube 3	0.25 mL	0.125 mL of Tube 2	X/27
Tube 4	0.25 mL	0.125 mL of Tube 3	X/81
Tube 5	0.25 mL	0.125 mL of Tube 4	X/243

### B. Rat/Mouse FGF-21 Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Using a pipette, reconstitute each of the Rat/Mouse FGF-21 Quality Control 1 and Quality Control 2 with 0.5 mL distilled or deionized water into the plastic vials. Invert and mix gently, let sit for 5 minutes then mix well.

### C. Matrix Solution Preparation

Reconstitute the EMTX-MSL with 0.5 mL of distilled or deionized water and let sit for 5 minutes. Vortex well and then add 0.5 mL Assay Buffer to the vial and vortex well.

## IX. ASSAY PROCEDURE

**NOTE: Please follow Assay Procedure carefully for correct samples. There are varying volumes added in Step 5 and Step 6 depending upon the sample type (rat or mouse).**

**Pre-warm all reagents to room temperature prior to setting up the assay.**

1. Dilute the 10X Wash Buffer concentrate 10 fold by mixing the entire content of each bottle of Wash Buffer with 450 mL deionized water (dilute both bottles with 900 mL deionized water).  
**Note: Hand wash only with multi-channel pipet. Do not use plate washer**
2. Remove the required number of strips from the Microtiter Assay Plate. Assemble the strips in an empty plate holder and wash each well 3 times with 300  $\mu$ L of diluted Wash Buffer per wash. Decant wash buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times. **Do not let wells dry before proceeding to the next step. Hand wash only with multi-channel pipet. Do not use automated plate washer**
3. Add in duplicate 20  $\mu$ L Matrix Solution to blank wells, Standard wells, and Quality Control wells.
4. Add in duplicate 20  $\mu$ L of Assay Buffer to blank wells.
5. Add in duplicate **30  $\mu$ L** of Assay Buffer to all sample wells for **Mouse Samples** or **20  $\mu$ L** of Assay Buffer to all sample wells for **Rat Samples**.
6. Add in duplicate 20  $\mu$ L Rat/Mouse FGF-21 Standards in the order of ascending concentration to the appropriate wells. Add in duplicate 20  $\mu$ L QC1 and 20  $\mu$ L QC2 to the appropriate wells. Add **10  $\mu$ L** of the unknown **mouse** samples in duplicate to the remaining wells or **20  $\mu$ L** of the unknown **rat** samples in duplicate to the remaining wells.
7. Add 10  $\mu$ L Detection Antibody to all wells. **For best result all additions should be completed within 30 minutes.** Cover the plate with plate sealer and incubate at room temperature for 2 hours on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400 to 500 rpm.
8. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.
9. Hand wash wells 3 times with diluted Wash Buffer, 300  $\mu$ L per well per wash. Decant and tap firmly after each wash to remove residual buffer.

## IX. ASSAY PROCEDURE (continued)

10. Add 100  $\mu$ L Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 minutes on the microtiter plate shaker.
11. Remove sealer, decant solutions from the plate, and tap plate to remove the residual fluid.
12. Hand wash wells 3 times with diluted Wash Buffer, 300  $\mu$ L per well per wash. Decant and tap firmly after each wash to remove residual buffer.
13. Add 100  $\mu$ L of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for **approximately** 5 to 20 minutes. Blue color should be formed in wells of the FGF-21 standards with intensity proportional to increasing concentrations of FGF-21.

**Note:** Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.

14. Remove sealer and add 100  $\mu$ L Stop Solution [**CAUTION: CORROSIVE SOLUTION**] and shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn to yellow after acidification. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. Read absorbance at 450 nm and 590 nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference of absorbance units. The absorbance of the highest FGF-21 standard should be approximately 2.0 - 3.0, or not to exceed the capability of the plate reader used.

**Note:** Mouse sample values must be multiplied by 2 for final FGF-21 concentrations.

**Assay Procedure - for Rat/Mouse FGF-21 ELISA kit (Cat. # EZRMFGF21-26K)**  
**Mouse Samples**

	Step 1	Step 2	Step 3	Step 5	Step 6	Step 7	Step 7-9	Step 10	Step 10-12	Step 13	Step 13	Step 14	Step 14	
Well #	Dilute each bottle of 10X Wash Buffer with 450mL Deionized Water.	Hand wash plate 3X with 300 $\mu$ L Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels	Matrix Solution	Assay Buffer	Standards/Controls Mouse Samples	Detection Ab	Seal, Agitate, Incubate 2 hours at Room Temperature. Hand wash 3X with 300 $\mu$ L Wash Buffer	Enzyme Solution	Seal, Agitate, Incubate 30 minutes at Room Temperature. Hand wash 3X with 300 $\mu$ L Wash Buffer	Substrate	Seal, Agitate, Incubate 5 - 20 minutes at Room Temperature.	Stop Solution	Read Absorbance at 450 nm and 590 nm.	
A1, B1			20 $\mu$ L	20 $\mu$ L	0 $\mu$ L	10 $\mu$ L		100 $\mu$ L		100 $\mu$ L				
C1, D1			20 $\mu$ L	0 $\mu$ L	20 $\mu$ L of Tube 5	↓		↓		↓		↓		↓
E1, F1			20 $\mu$ L	0 $\mu$ L	20 $\mu$ L of Tube 4									
G1, H1			20 $\mu$ L	0 $\mu$ L	20 $\mu$ L of Tube 3									
A2, B2			20 $\mu$ L	0 $\mu$ L	20 $\mu$ L of Tube 2									
C2, D2			20 $\mu$ L	0 $\mu$ L	20 $\mu$ L of Tube 1									
E2, F2			20 $\mu$ L	0 $\mu$ L	20 $\mu$ L of Reconstituted Standard									
G2, H2			20 $\mu$ L	0 $\mu$ L	20 $\mu$ L of QC 1									
A3, B3			20 $\mu$ L	0 $\mu$ L	20 $\mu$ L of QC 2									
C3, D3			0 $\mu$ L	30 $\mu$ L	10 $\mu$ L of Sample									
E3, F3			0 $\mu$ L	30 $\mu$ L	10 $\mu$ L of Sample									
G3, H3			0 $\mu$ L	30 $\mu$ L	10 $\mu$ L of Sample									
A4, B4 ↓			0 $\mu$ L	30 $\mu$ L	10 $\mu$ L of Sample									

**Assay Procedure - for Rat/Mouse FGF-21 ELISA kit (Cat. # EZRMFGF21-26K)**  
**Rat Samples**

	Step 1	Step 2	Step 3	Step 5	Step 6	Step 7	Step 7-9	Step 10	Step 10-12	Step 13	Step 13	Step 14	Step 14
Well #	Dilute each bottle of 10X Wash Buffer with 450mL Deionized Water.	Hand wash plate 3X with 300 $\mu$ L Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels	Matrix Solution	Assay Buffer	Standards/Controls Rat Samples	Detection Ab	Seal, Agitate, Incubate 2 hours at Room Temperature. Hand wash 3X with 300 $\mu$ L Wash Buffer	Enzyme Solution	Seal, Agitate, Incubate 30 minutes at Room Temperature. Hand wash 3X with 300 $\mu$ L Wash Buffer	Substrate	Seal, Agitate, Incubate 5 - 20 minutes at Room Temperature.	Stop Solution	Read Absorbance at 450 nm and 590 nm.
A1, B1			20 $\mu$ L	20 $\mu$ L	0 $\mu$ L	10 $\mu$ L		100 $\mu$ L		100 $\mu$ L			
C1, D1			20 $\mu$ L	0 $\mu$ L	20 $\mu$ L of Tube 5								
E1, F1			20 $\mu$ L	0 $\mu$ L	20 $\mu$ L of Tube 4								
G1, H1			20 $\mu$ L	0 $\mu$ L	20 $\mu$ L of Tube 3								
A2, B2			20 $\mu$ L	0 $\mu$ L	20 $\mu$ L of Tube 2								
C2, D2			20 $\mu$ L	0 $\mu$ L	20 $\mu$ L of Tube 1								
E2, F2			20 $\mu$ L	0 $\mu$ L	20 $\mu$ L of Reconstituted Standard								
G2, H2			20 $\mu$ L	0 $\mu$ L	20 $\mu$ L of QC 1								
A3, B3			20 $\mu$ L	0 $\mu$ L	20 $\mu$ L of QC 2								
C3, D3			0 $\mu$ L	20 $\mu$ L	20 $\mu$ L of Sample								
E3, F3			0 $\mu$ L	20 $\mu$ L	20 $\mu$ L of Sample								
G3, H3			0 $\mu$ L	20 $\mu$ L	20 $\mu$ L of Sample								
A4, B4 ↓			0 $\mu$ L	20 $\mu$ L	20 $\mu$ L of Sample								

## X. MICROTITER PLATE ARRANGEMENT

Rat/Mouse FGF-21 ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Tube 2	QC2	Etc.								
B	Blank	Tube 2	QC2	Etc.								
C	Tube 5	Tube 1	Sample 1									
D	Tube 5	Tube 1	Sample 1									
E	Tube 4	Reconstituted Standard	Sample 2									
F	Tube 4	Reconstituted Standard	Sample 2									
G	Tube 3	QC 1	Sample 3									
H	Tube3	QC 1	Sample 3									

## XI. CALCULATIONS

The dose-response curve of this assay fits best to a sigmoidal 4- or 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4- or 5-parameter logistic function

**Note:** All **Mouse sample calculated values must be multiplied by 2** to back-calculate the correct mathematical value.

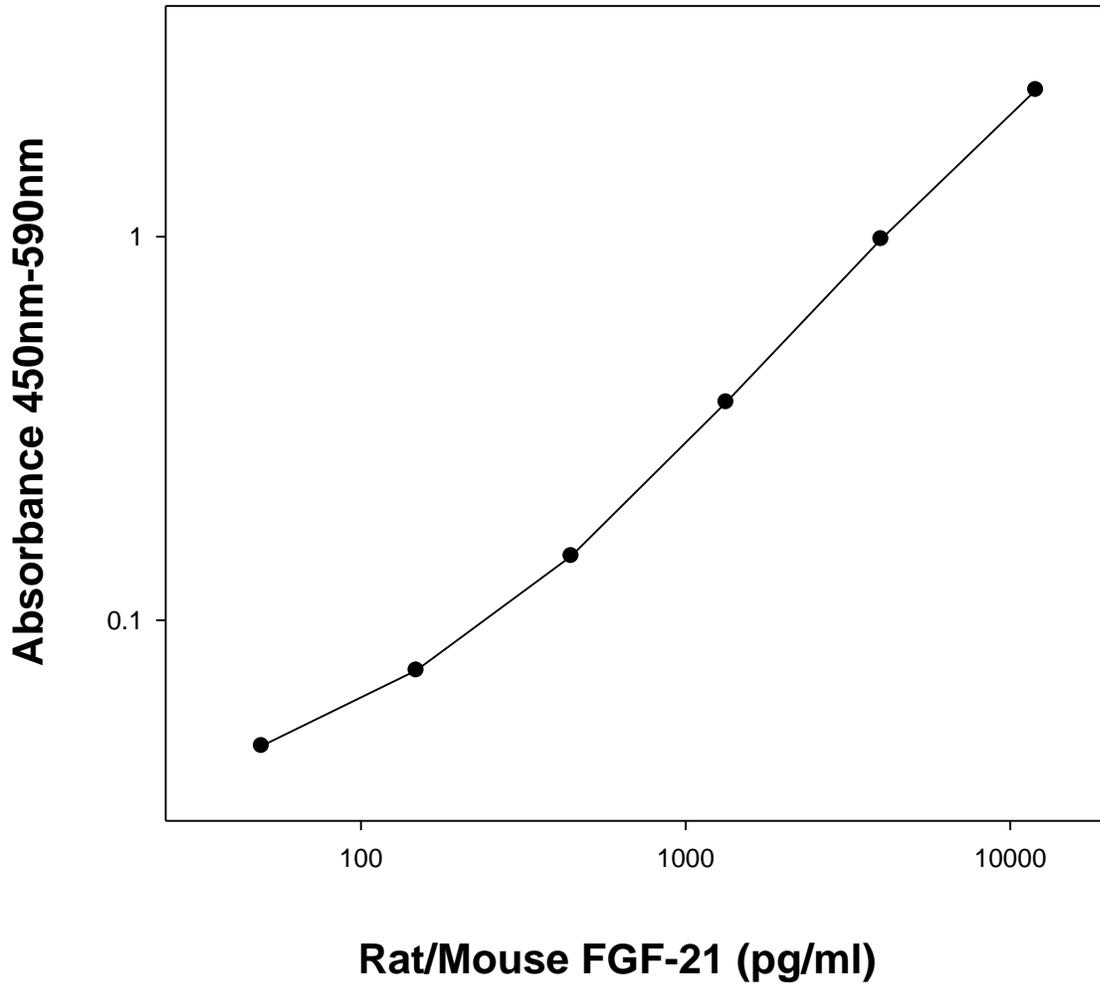
When sample volumes assayed differ from 10  $\mu\text{L}$  for Mouse and 20  $\mu\text{L}$  for Rat, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 10  $\mu\text{L}$  of rat sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 10  $\mu\text{L}$  for Mouse samples or 20  $\mu\text{L}$  for Rat samples, compensate the volume deficit with assay buffer.

## XII. INTERPRETATION

1. The assay will be considered accepted when all Quality Control values fall within the calculated Quality Control Range. If any QC's fall outside the control range, review results with a supervisor.
2. If the difference between duplicate results of a sample is  $>15\%$  CV, repeat the sample.
3. The limit of sensitivity of this assay is 10.0 pg/mL Rat/Mouse FGF-21 (10  $\mu\text{L}$  Mouse sample size or 20  $\mu\text{L}$  Rat sample size).
4. The appropriate range of this assay is 49.4 pg/mL to 12,000 pg/mL Rat/Mouse FGF-21 (10  $\mu\text{L}$  Mouse sample size or 20  $\mu\text{L}$  Rat sample size). Any result greater than 12,000 pg/mL in a 10  $\mu\text{L}$  Mouse sample or 20  $\mu\text{L}$  Rat sample, should be diluted using assay buffer and the assay repeated until the results fall within range.

### XIII. STANDARD CURVE

## Rat/Mouse FGF-21 ELISA Assay Typical Standard Curve



Typical Standard Curve, not to be used to calculate data.

## XIV. ASSAY CHARACTERISTICS

### A. Sensitivity

The lowest level of FGF-21 that can be detected by this assay is 10.0 pg/mL when using a 10  $\mu$ L Mouse sample size or 20  $\mu$ L Rat sample size.

### B. Specificity

The antibody pair used in this assay is specific to Rat/Mouse FGF-21 and does not cross-react to any of the Rat or Mouse endocrine hormones or cytokines tested.

Approximately 39% Cross-reactivity is observed to Human FGF-21

FGF-21 was detected in hamster and feline serum samples. However; no standards were used to properly calibrate cross-reactivity. FGF-21 was not detected in canine, guinea pig, rabbit, or porcine samples.

### C. Precision

Intra-Assay Variation

Sample No.	Mean FGF-21 Levels (pg/mL)	Intra-Assay % CV
1	302	9.1
2	872	5.8
3	1495	6.2
4	2782	3.2
5	7766	3.6
6	8813	2.7

The assay variations of EMD Millipore Rat/Mouse FGF-21 ELISA Kits were studied on four mouse serum samples and two rat serum samples with varying concentrations of endogenous FGF-21. The mean intra-assay variation was calculated from the results of eight replicate determinations in each assay for the indicated samples.

#### XIV. ASSAY CHARACTERISTICS (continued)

##### Inter-Assay Variation

Sample No.	Mean FGF-21 Levels (pg/mL)	Inter-Assay % CV
1	400	5.9
2	798	3.3
3	2096	4.7
4	3298	8.4
5	4174	8.3
6	5340	6.6

The assay variations of EMD Millipore Rat/Mouse FGF-21 ELISA Kits were studied on four mouse and two rat serum samples with varying concentrations of endogenous FGF-21. The mean inter-assay variations of each sample were calculated from the results of three separate assays with duplicate samples in each assay.

#### D. Recovery

##### Spike & Recovery of Rat/Mouse FGF-21 in Serum

Sample No.	FGF-21 Added pg/mL	Expected pg/mL	Observed pg/mL	% of Recovery
1	0	430	430	
	148.1	578	506	88
	444.4	874	739	85
	1333.3	1763	1307	74
2	0	442	442	
	148.1	590	602	102
	444.4	886	844	95
	1333.3	1775	1609	91
3	0	820	820	
	148.1	968	970	100
	444.4	1264	1232	97
	1333.3	2153	1865	87
4	0	2432	2432	
	148.1	2580	2539	98
	444.4	2876	2845	99
	1333.3	3765	3523	94
5	0	2897	2897	
	148.1	3045	3053	100
	444.4	3341	3351	100
	1333.3	4230	3979	94

Varying amounts of Rat/Mouse FGF-21 were added to three Mouse and two rat serum samples and the FGF-21 content was determined in two separate assays. The % of recovery = observed FGF-21 concentrations/expected FGF-21 concentrations x 100%.

#### XIV. ASSAY CHARACTERISTICS (continued)

##### E. Linearity and Dilution

Sample No.	Volume Sampled	Expected pg/mL	Observed pg/mL	% Of Expected
1	10	625	625	
	5	313	338	108
	2.5	156	172	110
	1.25	78	98	125
2	10	1290	1290	
	5	645	676	105
	2.5	323	301	93
	1.25	161	146	91
3	20	1007	1007	
	10	504	528	105
	5	252	266	106
	2.5	126	131	104
4	20	2099	2099	
	10	1050	1086	103
	5	525	536	102
	2.5	262	243	93

Two Mouse and two Rat serum samples with the indicated sample volumes were assayed in two separate experiments. Required amounts of matrix solution were added to compensate for lost volumes below 10  $\mu$ L (mouse) and 20  $\mu$ L (rat). The resulting dilution factors of 1.0, 2.0, 4.0, and 8.0 of sample volumes assayed, were applied in the calculation of observed FGF-21 concentrations. % expected = observed/expected x 100%.

## XV. QUALITY CONTROLS

The ranges for Quality Control 1 and 2 are provided on the card insert or can be located at the EMD Millipore website [emdmillipore.com](http://emdmillipore.com) using the catalog number as the keyword.

## XVI. TROUBLESHOOTING GUIDE

1. To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
3. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
4. Avoid cross contamination of any reagents or samples to be used in the assay.
5. Make sure all reagents and samples are added to the bottom of each well.
6. Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. Do not let the absorbency reading of the highest standard reach 3.0 units or higher after acidification.
9. High absorbance in background or blank wells could be due to 1) cross well contamination by standard solution or sample or 2) inadequate washing of wells with Wash Buffer or 3) overexposure to light after substrate has been added.

## XVII. REPLACEMENT REAGENTS

<b>Reagents</b>	<b>Cat. #</b>
Rat/Mouse FGF-21 ELISA Plate	EP26
10X HRP Wash Buffer Concentrate (50 mL)	EWB-HRP
Rat/Mouse FGF-21 Standards	E8026-K
Rat/Mouse FGF-21 Quality Controls 1 and 2	E6026-K
Matrix Solution	EMTX-MSL
Assay Buffer	EABPI
Rat/Mouse FGF-21 Detection Antibody	E1026
Enzyme Solution	EHRP
Substrate	ESS-TMB3
Stop Solution	ET-TMB

## **XVIII. ORDERING INFORMATION**

To place an order or to obtain additional information about our immunoassay products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

[emdmillipore.com/contact](http://emdmillipore.com/contact)

### **Conditions of Sale**

For Research Use Only. Not for Use in Diagnostic Procedures.

### **Safety Data Sheets (SDS)**

Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at [emdmillipore.com/msds](http://emdmillipore.com/msds).