User Guide

Rat/Mouse Fibroblast Growth Factor-21 (FGF-21) ELISA Kit

96-Well Plate

EZRMFGF21-26K

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.

Principles of Assay

This assay is a Sandwich ELISA based, sequentially, on:

- 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
- Binding of a second biotinylated polyclonal goat anti-FGF-21 antibody to the captured molecules
- Washing of unbound materials from samples
- Binding of streptavidin-horseradish peroxidase conjugate to the immobilized biotinylated antibodies
- Washing of excess free enzyme conjugates
- 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–590 nm 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.

Reagents Supplied

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

Note: Store all reagents at 2-8 °C

Reagents Supplied	Volume	Quantity	Cat. No.
Rat/Mouse FGF-21 ELISA Plate with 2 plate sealers **Note:** Unused strips should be resealed in the foil pouch with desiccant provided and stored at 2-8 °C.	-	1 plate 2 sealers	EP26
10X HRP Wash Buffer Concentrate 10X concentrate of 50 mM Tris Buffered Saline containing Tween® 20	50 mL	2 bottles	EWB-HRP
Rat/Mouse FGF-21 Standard	Lyophilized 0.5 mL	1 vial	E8026-K
Rat/Mouse FGF-21 Quality Controls 1 and 2	Lyophilized 0.5 mL	1 vial each	E6026-K
Matrix Solution	Lyophilized 0.5 mL	1 vial	EMTX-MSL
Assay Buffer 0.05 M PBS, pH 6.8, containing proprietary protease inhibitors, with Tween® 20, 0.08% Sodium azide and 1% BSA.	12 mL	1 bottle	EABPI
Rat/Mouse FGF-21 Detection Antibody	1.2 mL	1 bottle	E1026
Enzyme Solution	12 mL	1 bottle	EHRP
Substrate Solution 3,3’,5,5’-tetramethylbenzidine in buffer (light-sensitive, avoid unnecessary exposure to light)	12 mL	1 bottle	ESS-TMB3
Stop Solution 0.3 M HCl **Caution:** Corrosive Solution	12 mL	1 bottle	ET-TMB

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.

Reagent Precautions

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.

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 the next page.

Ingredient	Cat. No.	Full Label	
Human Fibroblast Growth Factor-21 Quality Controls 1 & 2	E6026-K		**Warning:** Harmful if swallowed. Toxic to aquatic life with long lasting effects. Avoid release to the environment.
Human Fibroblast Growth Factor-21 Standard	E8026-K		**Warning:** Harmful if swallowed. Toxic to aquatic life with long lasting effects. Avoid release to the environment.
Matrix Solution	EMTX-MSL		**Warning:** Harmful in contact with skin. May cause damage to organs Brain through prolonged or repeated exposure if swallowed. May cause damage to organ Brain through prolonged or repeated exposure if swallowed. Harmful to aquatic life with long lasting effects. Do not breathe dust/fume/gas/mist/vapors/ spray. Avoid release to the environment. Wear protective gloves/ protective clothing. IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/ doctor if you feel unwell. Get medical advice/ attention if you feel unwell. Wash contaminated clothing before reuse. Dispose of contents/ container to an approved waste disposal plant.

Ingredient	Cat. No.	Full Label	
Stop Solution	ET-TMB		**Warning:** May be corrosive to metals.
10X HRP 10X Wash Buffer Concentrate	EWB-HRP		**Warning:** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

Materials Required (Not Provided)

- Multi-channel Pipettes and pipette tips: 5 µL-50 µL and 50 µL-300 µL
- Pipettes and pipette tips: 10 µL-20 µL or 20 µL-100 µL
- Buffer and Reagent Reservoirs
- Vortex Mixer
- De-ionized water
- Microtiter Plate Reader capable of reading absorbency at 450 nm
- Orbital Microtiter Plate Shaker
- Absorbent Paper or Cloth

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 3,000 x *g* for 15 minutes at 4 ±2 °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 ≤ –20 °C for later use. For long-term storage, keep at −70 °C. Avoid freeze/thaw cycles.
5. To prepare plasma samples, whole blood should be collected into centrifuge tubes containing enough K3EDTA 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.

Reagent Preparation

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 ≤ −20 °C. Avoid multiple freeze/thaw cycles.

Tube #	Volume of Deionized Water to Add	Volume of Standard to Add	Standard Stock Concentration
Reconstituted standard	0.5 mL	0	X (refer to analysis sheet for exact concentration)

Tube #	Volume of Assay Running Buffer (EARB-6) 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

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 vials. Invert and mix gently, let sit for 5 minutes then mix well.

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.

Assay Procedure

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 µL 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 µL Matrix Solution to blank wells, Standard wells, and Quality Control wells.

4. Add in duplicate 20 µL of Assay Buffer to blank wells.

5. Add in duplicate 30 µL of Assay Buffer to all sample wells for Mouse Samples or 20 µL of Assay Buffer to all sample wells for Rat Samples.

6. Add in duplicate 20 µL Rat/Mouse FGF-21 Standards in the order of ascending concentration to the appropriate wells. Add in duplicate 20 µL QC1 and 20 µL QC2 to the appropriate wells. Add 10 µL of the unknown mouse samples in duplicate to the remaining wells or 20 µL of the unknown rat samples in duplicate to the remaining wells.

7. Add 10 µL 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 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.

10. Add 100 µL 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 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.

13. Add 100 µL 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 µL 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

Mouse Samples

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

Assay Procedure for Rat/Mouse FGF-21 ELISA Kit

Rat Samples

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

Microtiter Plate Arrangement

Rat/Mouse FGF-21 ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Tube 2	QC 2	Etc.								
B	Blank	Tube 2	QC 2	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	Tube 3	QC 1	Sample 3									

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.

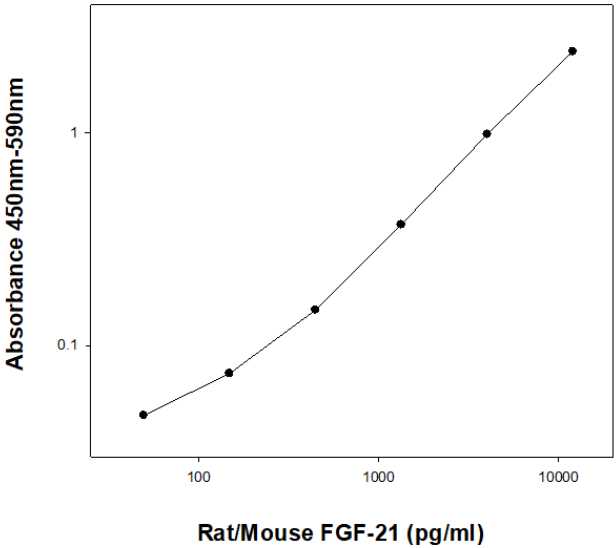
When sample volumes assayed differ from 10 µL for Mouse and 20 µL for Rat, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (for example, if 10 µL of rat sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 10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

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 µL Mouse sample size or 20 µL 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 µL Mouse sample size or 20 µL Rat sample size). Any result greater than 12,000 pg/mL in a 10 µL Mouse sample size or 20 µL Rat sample size should be diluted using assay buffer, and the assay repeated until the results fall within range.

Standard Curve

Rat/Mouse FGF-21 ELISA Assay Typical Standard Curve



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

Assay Characteristics

Sensitivity

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

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.

Precision

Intra-Assay Variation

	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 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.

Inter-Assay Variation

	Mean FGF-21 Levels (pg/mL)	Intra-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 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.

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%.

Linearity of Sample 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 µL (mouse) and 20 µL (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%.

Quality Controls

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert, or available at our website SigmaAldrich.com.

Troubleshooting

- 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.
- Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
- 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.
- Avoid cross contamination of any reagents or samples to be used in the assay.
- Make sure all reagents and samples are added to the bottom of each well.
- 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.
- Remove any air bubbles formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
- Do not let the absorbency reading of the highest standard reach 3.0 units or higher after acidification.
- High absorbance in background or blank wells could be due to:
    - cross well contamination by standard solution or sample, or
    - inadequate washing of wells with Wash Buffer, or
    - overexposure to light after substrate has been added

Product Ordering

Products are available for online ordering at SigmaAldrich.com.

Replacement Reagents

Reagents	Cat. No.
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

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