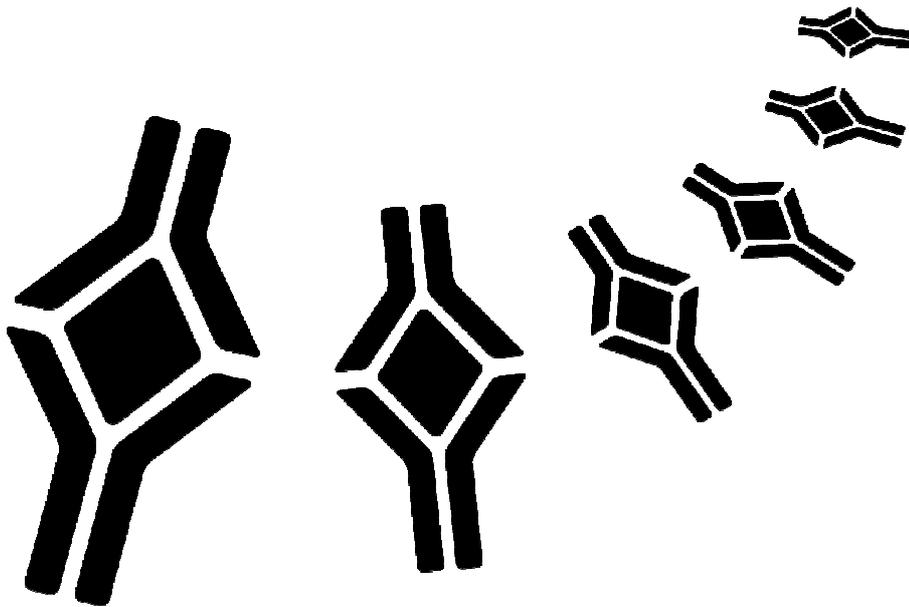


**BioVendor**

Research  
and Diagnostic Products



## MOUSE AND RAT FGF-21 ELISA

Product Data Sheet

Cat. No.: RD291108200R

For Research Use Only

# CONTENTS

---

1.	INTENDED USE	3
2.	STORAGE, EXPIRATION	3
3.	INTRODUCTION	4
4.	TEST PRINCIPLE	5
5.	PRECAUTIONS	5
6.	TECHNICAL HINTS	6
7.	REAGENT SUPPLIED	6
8.	MATERIAL REQUIRED BUT NOT SUPPLIED	7
9.	PREPARATION OF REAGENTS	7
10.	PREPARATION OF SAMPLES	10
11.	ASSAY PROCEDURE	11
12.	CALCULATIONS	13
13.	PERFORMANCE CHARACTERISTICS	14
14.	DEFINITION OF THE STANDARD	17
15.	METHOD COMPARISON	17
16.	TROUBLESHOOTING AND FAQs	18
17.	REFERENCES	19
18.	EXPLANATION OF SYMBOLS	20

**»» This kit is manufactured by:  
BioVendor – Laboratorní medicína a.s.**

**»» Use only the current version of Product Data Sheet enclosed with the kit!**

## 1. INTENDED USE

---

The RD291108200R Mouse and Rat FGF-21 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of mouse and/or rat FGF-21 (fibroblast growth factor-21).

### »» Features

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures FGF-21 in serum
- Assay format is 96 wells
- Quality Controls are mouse and rat serum based. No human sera are used
- Standard is mouse recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

## 2. STORAGE, EXPIRATION

---

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

### 3. INTRODUCTION

---

The fibroblast growth factor family (FGFs) are a family of more than 20 small (17-26 kDa) secreted peptides. The initial characterisation of these proteins focused on their ability to stimulate fibroblast proliferation through FGF receptors (FGFRs). Members of FGFs family play important roles in defining and regulating the development and function of endocrine tissues as well as modulating various metabolic processes.

A recently described member of FGFs family, FGF-21, also called Fibroblast growth factor 21 precursor and UNQ3115/PRO10196, has been characterised as a potent metabolic regulator. FGF-21 is preferentially expressed in liver and regulates glucose uptake in fat cells. Moreover, therapeutic administration of FGF-21 decreased plasma glucose levels and triglycerides to near normal levels in multiple mouse models of type 2 diabetes. Short-term treatment of normal or db/db mice with FGF-21 lowered plasma levels of insulin and improved glucose clearance compared with vehicle after oral glucose tolerance testing. Constant infusion of FGF-21 for 8 weeks in db/db mice nearly normalized fed blood glucose levels and increased plasma insulin levels. When administered daily for 6 weeks to diabetic rhesus monkeys, FGF-21 caused dramatic decline in fasting plasma glucose, fructosamine, triglycerides, insulin, and glucagon. FGF-21 administration also led to significant improvements in lipoprotein profiles, including lowering of low-density lipoprotein cholesterol and raising of high-density lipoprotein cholesterol as well as beneficial changes in the circulating levels of several cardiovascular risk factors.

FGF-21, when overexpressed, protected animals from diet-induced obesity. These results define a functional role for FGF-21 in vivo and provide evidence that FGF-21 can lower glucose and triglyceride levels in diabetic animals.

In contrast to several members of the FGF family which may induce therapeutically undesirable in vivo proliferation of various cell types, a recent study demonstrated that FGF-21 did not induce mitogenicity, hypoglycemia or weight gain at any dose tested in diabetic or healthy animals or when overexpressed in transgenic mice. Thus, FGF-21 appears to have considerable potential for the treatment of diabetes mellitus.

#### Areas of investigation:

Lipid metabolism

Diabetes mellitus type 2

Metabolic syndrome

## 4. TEST PRINCIPLE

---

In the BioVendor Mouse and Rat FGF-21 ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-mouse FGF-21 antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-mouse FGF-21 antibody is added and incubated for 60 minutes with captured FGF-21. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of FGF-21. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

## 5. PRECAUTIONS

---

- **For professional use only**
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit contains components of animal origin. These materials should be handled as potentially infectious
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

## 6. TECHNICAL HINTS

---

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

## 7. REAGENT SUPPLIED

---

<i>Kit Components</i>	<i>State</i>	<i>Quantity</i>
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody Conc. (10x)	concentrated	1.3 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Quality Control MOUSE	lyophilized	2 vials
Quality Control RAT	lyophilized	2 vials
Dilution Buffer	ready to use	20 ml
Biotin-Ab Diluent	ready to use	13 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml
Product Data Sheet + Certificate of Analysis	-	1 pc

## 8. MATERIAL REQUIRED BUT NOT SUPPLIED

---

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 5-1000  $\mu$ l with disposable tips
- Multichannel pipette to deliver 100  $\mu$ l with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtiter plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with  $450 \pm 10$  nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650nm)
- Software package facilitating data generation and analysis (optional)

## 9. PREPARATION OF REAGENTS

---

- All reagents need to be brought to room temperature prior to use
- Always prepare only the appropriate quantity of reagents for your test
- Do not use components after the expiration date marked on their label

- Assay reagents supplied ready to use:

### **Antibody Coated Microtiter Strips**

#### Stability and storage:

Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

### **Streptavidin-HRP Conjugate**

#### **Dilution Buffer**

#### **Biotin-Ab Diluent**

#### **Substrate Solution**

#### **Stop Solution**

#### Stability and storage:

Opened reagents are stable 3 months when stored at 2-8°C.

- **Assay reagents supplied concentrated or lyophilized:**

### **FGF-21 Master Standard**

**Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!**

Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the FGF-21 in the stock solution is **2560 pg/ml (U/ml)**. See chapter 14. Definition of Standard.

For measurement of **mouse samples**, prepare set of standards using Dilution Buffer as follows:

<i>Volume of Standard</i>	<i>Dilution Buffer</i>	<i>Concentration</i>
Stock	-	2560 pg/ml
250 µl of stock	250 µl	1280 pg/ml
250 µl of 1280 pg/ml	250 µl	640 pg/ml
250 µl of 640 pg/ml	250 µl	320 pg/ml
250 µl of 320 pg/ml	250 µl	160 pg/ml
250 µl of 160 pg/ml	250 µl	80 pg/ml
250 µl of 80 pg/ml	250 µl	40 pg/ml

For measurement of **rat samples**, prepare set of standards using Dilution Buffer as follows:

<i>Volume of Standard</i>	<i>Dilution Buffer</i>	<i>Concentration</i>
Stock	-	2560 U/ml
250 µl of stock	250 µl	1280 U/ml
250 µl of 1280 U/ml	250 µl	640 U/ml
250 µl of 640 U/ml	250 µl	320 U/ml
250 µl of 320 U/ml	250 µl	160 U/ml
250 µl of 160 U/ml	250 µl	80 U/ml
250 µl of 80 U/ml	250 µl	40 U/ml

**Prepared Standards are ready to use, do not dilute them.**

#### Stability and storage:

The reconstituted Standard stock solution must be used immediately or aliquoted and frozen at -20 °C for 3 months. Avoid repeating freezing/thawing cycles.

**Do not store the diluted Standard solutions.**

### **Quality Controls MOUSE/ RAT**

**Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!**

Reconstitute appropriate Quality Control (MOUSE or RAT) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

**Reconstituted Quality Controls are ready to use, do not dilute them.**

#### Stability and storage:

The reconstituted Quality Controls must be used immediately or aliquoted and frozen at -20°C for 3 months. Avoid repeated freeze/thaw cycles.

**Do not store the diluted Quality Controls.**

#### Note:

*Concentration of analyte in Quality Control need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Control serves just for control that the kit works in accordance with PDS and CoA and that ELISA test was carried out properly.*

### **Biotin Labelled Antibody Conc. (10x)**

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (10x) with 9 parts Biotin-Ab Diluent. Example: 100 µl of Biotin Labelled Antibody Concentrate (10x) + 900 µl of Biotin-Ab Diluent for 1 strip (8 wells).

#### Stability and storage:

Opened Biotin Labelled Antibody Concentrate (10x) is stable 3 months when stored at 2-8°C.

**Do not store the diluted Biotin Labelled Antibody solution.**

### **Wash Solution Conc. (10x)**

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 ml of Wash Solution Concentrate (10x)+ 900 ml of distilled water for use of all 96-wells.

#### Stability and storage:

The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

## 10. PREPARATION OF SAMPLES

---

The kit measures FGF-21 in serum.

Samples should be assayed immediately after collection or should be stored at  $-20^{\circ}\text{C}$ . Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

### **Sample dilution**

The FGF-21 levels may depend on feeding, diurnal cycle, pathophysiology and strain of the individual. Suitable sample dilution should be tested by the researcher in advance. It is recommended to run 2-3 samples with dilution 3x, 10x and 20x to choose a suitable dilution for all samples.

#### Dilution 3x

Dilute samples with the Dilution Buffer just prior to the assay, e.g. 50  $\mu\text{l}$  of sample + 100  $\mu\text{l}$  of Dilution Buffer for singlets, or preferably 100  $\mu\text{l}$  of sample + 200  $\mu\text{l}$  of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

#### Dilution 10x

Dilute samples with the Dilution Buffer just prior to the assay, e.g. 15  $\mu\text{l}$  of sample + 135  $\mu\text{l}$  of Dilution Buffer for singlets, or preferably 30  $\mu\text{l}$  of sample + 270  $\mu\text{l}$  of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

#### Dilution 20x

Dilute samples 20x with the Dilution Buffer just prior to the assay, e.g. 7  $\mu\text{l}$  of sample + 133  $\mu\text{l}$  of Dilution Buffer for singlets, or preferably 14  $\mu\text{l}$  of sample + 266  $\mu\text{l}$  of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

#### Stability and storage:

Samples should be stored at  $-20^{\circ}$ , or preferably at  $-70^{\circ}\text{C}$  for long-term storage. Avoid repeated freeze/ thaw cycles.

### **Do not store the diluted samples.**

See Chapter 13 for stability of serum samples when stored at  $2-8^{\circ}\text{C}$  and effect of freezing/thawing on the concentration of FGF-21.

*Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.*

## 11. ASSAY PROCEDURE

---

1. Pipet **100 µl** of Standards, Quality Control, Dilution Buffer (=Blank) and diluted samples, preferably in duplicates, into the appropriate wells. See *Figure 1* and *Figure 2* for examples of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add **100 µl** of Biotin Labelled Antibody solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µl** of Streptavidin-HRP Conjugate solution into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 min.**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for **10 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 – 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm.  
**The absorbance should be read within 5 minutes following step 12.**

*Note: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine FGF-21 concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were “in range” at 450 nm.*

*Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.*

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
<b>A</b>	<b>2560 pg/ml</b>	<b>QC MOUSE</b>	Sample 9	Sample 17	Sample 25	Sample 33
<b>B</b>	<b>1208 pg/ml</b>	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
<b>C</b>	<b>640 pg/ml</b>	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
<b>D</b>	<b>320 pg/ml</b>	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
<b>E</b>	<b>160 pg/ml</b>	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
<b>F</b>	<b>80 pg/ml</b>	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
<b>G</b>	<b>40 pg/ml</b>	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
<b>H</b>	<b>Blank</b>	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40

Figure 1: Example of a work sheet for Mouse FGF-21 ELISA.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
<b>A</b>	<b>2560 U/ml</b>	<b>QC RAT</b>	Sample 9	Sample 17	Sample 25	Sample 33
<b>B</b>	<b>1208 U/ml</b>	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
<b>C</b>	<b>640 U/ml</b>	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
<b>D</b>	<b>320 U/ml</b>	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
<b>E</b>	<b>160 U/ml</b>	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
<b>F</b>	<b>80 U/ml</b>	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
<b>G</b>	<b>40 U/ml</b>	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
<b>H</b>	<b>Blank</b>	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40

Figure 2: Example of a work sheet for Rat FGF-21 ELISA.

## 12. CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm.

Results are reported as concentration of FGF-21 **pg/ml in mouse samples**.

Results are reported as concentration of FGF-21 **U/ml in rat samples**.

Alternatively, the logit log function can be used to linearize the standard curve, i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.

**The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay.**

**EXAMPLE for 3x diluted rat samples: 160 U/ml (from standard curve) x 3 (dilution factor) = 480 U/ml.**

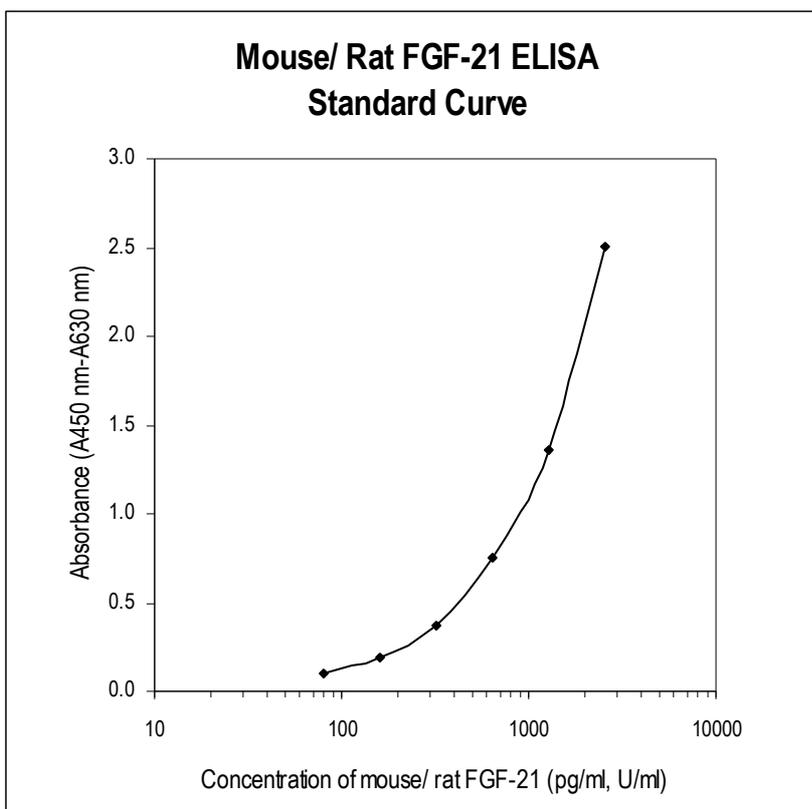


Figure 3: Typical Standard Curve for Mouse and Rat FGF-21 ELISA.

## 13. PERFORMANCE CHARACTERISTICS

---

➤➤ **Typical analytical data of BioVendor Mouse and Rat FGF-21 ELISA are presented in this chapter**

- **Sensitivity**

Limit of Detection (LOD), defined as concentration of analyte giving absorbance higher than mean absorbance of blank\* plus three standard deviations of the absorbance of blank:  $A_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$ , is calculated from the real FGF-21 values in wells and is 18.4 pg/ml (U/ml).

\*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. The final concentration of samples calculated from the standard curve must be multiplied by the respective dilution factor.

- **Specificity**

Sera of several mammalian species were measured in the assay. See results below.

For details please contact us at [info@biovendor.com](mailto:info@biovendor.com).

<i>Mammalian serum sample</i>	<i>Observed crossreactivity</i>
Bovine	yes
Cat	yes
Dog	yes
Goat	yes
Hamster	yes
Horse	no
Human	no
Monkey	no
Pig	yes
Rabbit	no
Sheep	yes

➤➤ Presented results are multiplied by respective dilution factor

• **Precision**

Intra-assay (Within-Run) (n=8)

<i>Sample</i>	<i>Mean</i>	<i>SD</i>	<i>CV (%)</i>
mouse 1 (ng/ml)	1.62	0.16	9.9
mouse 2 (ng/ml)	3.31	0.23	6.9
rat 1 (U/ml)	693	65.7	9.5
rat 2 (U/ml)	392	29.1	7.4

Inter-assay (Run-to-Run) (n=6)

<i>Sample</i>	<i>Mean</i>	<i>SD</i>	<i>CV (%)</i>
mouse 1 (ng/ml)	11.50	0.99	8.7
rat 2 (U/ml)	1338.4	80.77	6.0

• **Spiking Recovery**

Serum samples were spiked with different amounts of mouse or rat FGF-21 and assayed.

<i>Sample</i>	<i>Observed</i>	<i>Expected</i>	<i>Recovery O/E (%)</i>
mouse 1 (ng/ml)	1.49	-	-
	6.91	7.89	87.5
	5.31	4.69	113.2
	3.25	3.09	105.2
mouse 2 (ng/ml)	3.78	-	-
	10.48	10.2	102.8
	7.35	7.0	105.0
rat 1 (U/ml)	4.57	5.40	84.7
	208	-	-
	1374	1168	117.6
rat 2 (U/ml)	691	688	100.3
	494	448	110.1
	438	-	-
rat 2 (U/ml)	1429	1398	102.2
	913	918	99.4
	660	678	97.3

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed	Expected	Recovery O/E (%)
mouse 1 (ng/ml)	-	18.69	-	-
	2x	7.96	9.34	85.2
	4x	4.96	4.67	106.2
	8x	2.18	2.34	93.4
mouse 2 (ng/ml)	-	4.53	-	-
	2x	2.30	2.26	101.7
	4x	1.22	1.13	107.8
	8x	0.50	0.57	104.3
rat 1 (U/ml)	-	1080	-	-
	2x	602	540	111.5
	4x	274	270	101.4
	8x	126	135	93.7
rat 2 (U/ml)	-	1209	-	-
	2x	655	604	108.4
	4x	336	302	111.2
	8x	145	151	95.9

- **Stability of samples stored at 2-8°C**

Samples should be stored at -20°C. However, no decline in concentration of FGF-21 was observed in mouse and rat serum samples after 7 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and thimerosal, resulting in the final concentration of 0.03% and 0.05%, respectively.

Sample	Incubation Temp, Period	Serum
mouse 1 (ng/ml)	-20°C	1.05
	2-8°C, 1 day	0.97
	2-8°C, 7 days	1.10
mouse 2 (ng/ml)	-20°C	1.09
	2-8°C, 1 day	1.11
	2-8°C, 7 days	1.11
rat 1 (U/ml)	-20°C	542
	2-8°C, 1 day	563
	2-8°C, 7 days	451
rat 2 (U/ml)	-20°C	956
	2-8°C, 1 day	992
	2-8°C, 7 days	983

- **Effect of Freezing/Thawing**

No decline was observed in concentration of mouse and rat FGF-21 in serum samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	Number of f/t cycles	Serum
mouse 1 (ng/ml)	1x	1.17
	3x	1.24
	5x	1.15
mouse 2 (ng/ml)	1x	1.27
	3x	1.15
	5x	1.20
rat 1 (U/ml)	1x	619
	3x	588
	5x	573
rat 2 (U/ml)	1x	1054
	3x	1089
	5x	1044

#### 14. DEFINITION OF THE STANDARD

---

Standard used in this assay is mouse recombinant protein based. As there is no rat recombinant FGF-21 protein available, mouse recombinant is used as a standard for quantification of FGF-21 in rat samples and concentration in those samples is determine in U/ml.

Standard is recombinant *E.coli* expressed protein consisting of 198 AA. Calculated molecular weight is 21.2 kDa.

#### 15. METHOD COMPARISON

---

Mouse and Rat FGF-21 ELISA was not compared to any other immunoassay.

## 16. TROUBLESHOOTING AND FAQs

---

### »» Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

### »» High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

### »» High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

## 17. REFERENCES

---

### »» References to mouse/rat FGF-21:

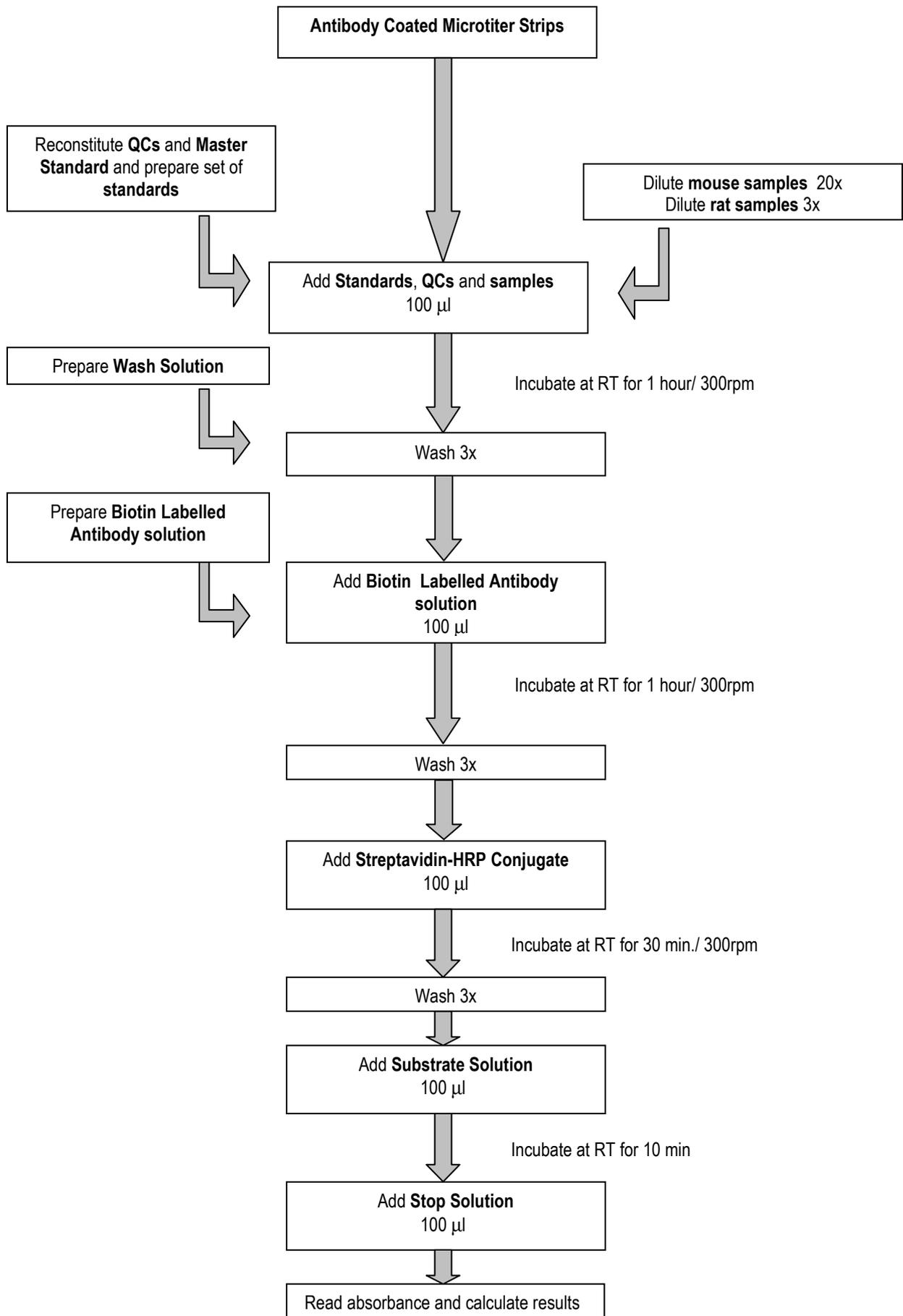
- Chen WW, Li L, Yang GY, Li K, Qi XY, Zhu W, Tang Y, Liu H, Boden G.: Circulating FGF-21 Levels in Normal Subjects and in Newly Diagnose Patients with Type 2 Diabetes Mellitus. *Exp Clin Endocrinol Diabetes*. 2008 Jan;116(1):65-8.
- Kharitonov A, Shanafelt AB.: Fibroblast growth factor-21 as a therapeutic agent for metabolic diseases. *BioDrugs*. 2008;22(1):37-44.
- Kharitonov A, Dunbar JD, Bina HA, Bright S, Moyers JS, Zhang C, Ding L, Micanovic R, Mehrbod SF, Knierman MD, Hale JE, Coskun T, Shanafelt AB.: FGF-21/FGF-21 receptor interaction and activation is determined by betaKlotho. *J Cell Physiol*. 2008 215(1):1-7.
- Suzuki M, Uehara Y, Motomura-Matsuzaka K, Oki J, Koyama Y, Kimura M, Asada M, Komi-Kuramochi A, Oka S, Imamura T.: {beta}Klotho is required for FGF21 signaling FGFR1c and FGFR3c. *Mol Endocrinol*. 2008
- Zhang X, Yeung DC, Karpisek M, Stejskal D, Zhou ZG, Liu F, Wong RL, Chow WS, Tso AW, Lam KS, Xu A.: Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. *Diabetes*. 2008 Feb 5
- Kharitonov A., Wroblewski V.J., Koester A., Chen Y-F., Clutinger C.K., Tigno X.T., Hansen B.C., Shanafelt A.B. and Etgen G.J.: The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. *Endocrinology* 148(2):774-81(2007)
- Moore D.D.: Physiology. Sister act. *Science* 316(5830):1436-8 (2007)
- Ogawa Y., Kurosu H., Yamamoto M., Nandi A., Rosenblatt K.P., Goetz R., Eliseenkova A.V., Mohammadi M. and Kuro-o M.: BetaKlotho is required for metabolic activity of fibroblast growth factor 21. *Proc Natl Acad Sci U S A*. 104(18):7432-7 (2007)
- Wente W., Efanov A.M., Brenner M., Kharitonov A., Koster A., Sandusky G.E., Sewing S., Treinies I., Zitzer H. and Gromada J.: Fibroblast Growth Factor-21 Improves Pancreatic  $\beta$ -cells Function and Survival by Activation of Extracellular Signal-Regulated Kinase 1/2 and Akt Signaling Pathway.: *Diabetes*, Vol 55:2470-2478(2006)
- Kharitonov A., Shiyanova L.T., Koester A., Ford A.M., Micanovic R., Galbreath E.J., Sandusky G.E., Hammond L.J., Moyers J.S., Owens R.A., Gromada J., Brozinick J.T., Hawkins E.D., Wroblewski V.J., Li D-S, Mehrbod F., Jaskunas S.R. and Shanafelt A.B.: FGF-21 as a novel metabolic regulator. *J Clin Invest*.115:1627-1635 (2005)

»» For more references on this product see our WebPages at [www.biovendor.com](http://www.biovendor.com)

## 18. EXPLANATION OF SYMBOLS

	Catalogue number
	Content
	Lot number
	Attention, see instructions for use
	Potential biological hazard
	Expiry date
	Storage conditions
	Name and registered office of the manufacturer

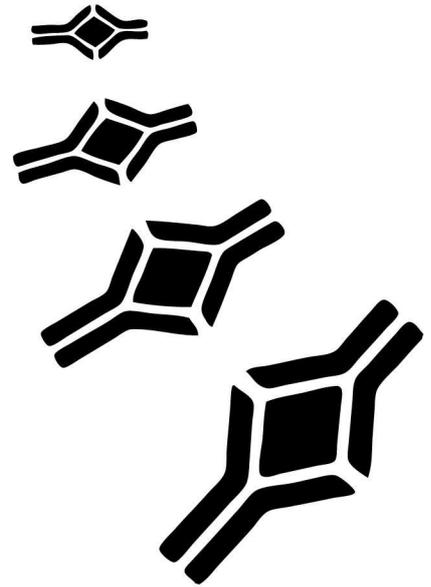
## Assay Procedure Summary



12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>	<b>G</b>	<b>H</b>

**NOTES**





---

**BioVendor – Laboratorní medicína a.s.**

Karasek 1767/1, 621 00 Brno, Czech Republic

Phone: +420-549-124-185, Fax: +420-549-211-460

E-mail: [info@biovendor.com](mailto:info@biovendor.com), [sales@biovendor.com](mailto:sales@biovendor.com)

Web: [www.biovendor.com](http://www.biovendor.com)

There are BioVendor branches and distributors near you.

To find the office closest to you, visit [www.biovendor.com/contact](http://www.biovendor.com/contact)

