Version 3 Last updated 6 May 2022

# ab223587
# Mouse/Rat FGF1 SimpleStep ELISA® Kit

For the quantitative measurement of FGF1 protein in mouse and rat plasma, serum, cell culture supernatant, urine, and cell and tissue extract samples.

This product is for research use only and is not intended for diagnostic use.

# Table of Contents

# 1. Overview

FGF1 *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of FGF1 protein in mouse/rat plasma, serum, cell culture supernatant, urine, and cell and tissue extract samples.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB Development Solution is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

FGF1, also known as Fibroblast Growth Factor 1 or FGF acidic, is an important activator for several signaling cascades with roles in the regulation of cell survival, cell division, angiogenesis, cell differentiation and cell migration. It functions as potent mitogen in vitro. In the presence of heparin, FGF1 binds in a 1:2 stoichiometry with FGF1 receptor protein, causing the autophosphorylation and activation of the receptor. This complex then binds to integrin and recruits PTPN11 to begin FGF1 signaling. Because of its possible role in organ development and in tumor growth, FGF1 is an important subject of study for the treatment of diabetes and the diagnosis and treatment of cancer. Mouse and rat FGF1 are identical on an amino acid level.

## 2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells

Add 50 µL Antibody Cocktail to all wells

Incubate at room temperature for 1 hour

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

Add 100 µL TMB Development Solution to each well and incubate for 10 minutes.

Add 100 µL Stop Solution and read OD at 450 nm

## 3. Precautions

**Please read these instructions carefully prior to beginning the assay.**

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

## 4. Storage and Stability

**Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.**

Refer to list of materials supplied for storage conditions of individual components.

## 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. Materials Supplied

| Item | Quantity | Storage Condition |
|---|---|---|
| Mouse/Rat FGF1 Capture Antibody 10X | 600 µL | +4°C |
| Mouse/Rat FGF1 Detector Antibody 10X | 600 µL | +4°C |
| Mouse/Rat FGF1 Lyophilized Recombinant Protein | 2 Vials | +4°C |
| Antibody Diluent CPR | 6 mL | +4°C |
| Wash Buffer PT 10X | 20 mL | +4°C |
| Cell Extraction Buffer PTR 5X | 10 mL | +4°C |
| Cell Extraction Enhancer Solution 50X | 1 mL | +4°C |
| TMB Development Solution | 12 mL | +4°C |
| Stop Solution | 12 mL | +4°C |
| Sample Diluent NS | 50 mL | +4°C |
| Sample Diluent 25BS | 20 mL | +4°C |
| Anti-tag coated microplate (12 x 8 well strips) | 96 Wells | +4°C |
| Plate Seal | 1 | +4°C |

## 7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

## 8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.

- The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.
- To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

# 9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.
- Sample Diluent 25BS may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

## 9.1 1X Cell Extraction Buffer PTR (For cell and tissue extracts only):

Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL Cell Extraction Buffer PTR 5X and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently. If required protease inhibitors can be added.

Alternative – Enhancer may be added to 1X Cell Extraction Buffer PTR after extraction of cells or tissue. Refer to note in the Troubleshooting section.

## 9.2 1X Wash Buffer PT:

Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

## 9.3 Antibody Cocktail:

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPR. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent CPR. Mix thoroughly and gently.

## 10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the FGF1 lyophilized standard by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the FGF1 lyophilized standard by adding 500 µL Diluent. Hold at room temperature for 10 minutes and mix gently. This is the 6,000 pg/mL **Stock Standard** Solution.

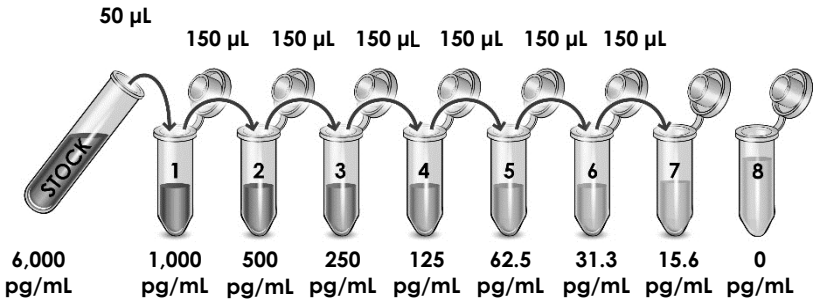
10.2 Label eight tubes, Standards 1– 8.

10.3 For **cell and tissue extract sample measurements**, reconstitute the FGF1 lyophilized standard with 1X Cell Extraction Buffer PTR. Add 250 µL 1X Cell Extraction Buffer PTR into tube number 1 and 150 µL of 1X Cell Extraction Buffer PTR into numbers 2-8.

For **serum, plasma and cell culture supernatant sample measurements**, reconstitute the FGF1 lyophilized standard with Sample Diluent NS. Add 250 µL Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.

For **urine sample measurements**, reconstitute the FGF1 lyophilized standard with Sample Diluent 25BS. Add 250 µL Sample Diluent 25BS into tube number 1 and 150 µL of Sample Diluent 25BS into numbers 2-8.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



# 11. Sample Preparation

| Typical Sample Dynamic Range | |
|---|---|
| **Sample Type** | **Range** |
| Mouse Brain Tissue Extract | 1.9-31 µg/ml |
| Rat Brain Tissue Extract | 1.9-63 µg/ml |
| Mouse Urine | 3.1-25% |
| Rat Urine | 3.1-50% |
| Mouse/Rat Plasma - Citrate | 1.6-25% |
| Mouse/Rat Plasma - EDTA | 1.6-50% |
| Mouse Serum | 1.6-50% |
| Rat Serum | 1.6-25% |
| RAW 264.7 Supernatant | 5.9-100% |

## 11.1 Plasma:

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute mouse and rat EDTA plasma at least two-fold into Sample Diluent NS and assay. Dilute mouse and rat citrate plasma samples at least four-fold into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

## 11.2 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute mouse serum samples at least two-fold into Sample Diluent NS and assay. Dilute rat serum samples at least four-fold into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

## 11.3 Cell Culture Supernatants:

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

### 11.4 Urine:

Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Dilute mouse urine at least four-fold in Sample Diluent 25BS and assay. Dilute rat urine at least two-fold in Sample Diluent 25BS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

### 11.5 Preparation of extracts from cell pellets:

11.5.1 Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C.

11.5.2 Rinse cells twice with PBS.

11.5.3 Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR.

11.5.4 Incubate on ice for 20 minutes.

11.5.5 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.5.6 Transfer the supernatants into clean tubes and discard the pellets.

11.5.7 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.5.8 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

### 11.6 Preparation of extracts from adherent cells by direct lysis (alternative protocol):

11.6.1 Remove growth media and rinse adherent cells 2 times in PBS.

11.6.2 Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate).

11.6.3 Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes.

11.6.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.6.5 Transfer the supernatants into clean tubes and discard the pellets.

11.6.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.6.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

## 11.7 Preparation of extracts from tissue homogenates:

11.7.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

11.7.2 Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly.

11.7.3 Incubate on ice for 20 minutes.

11.7.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.7.5 Transfer the supernatants into clean tubes and discard the pellets.

11.7.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.7.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

## 12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

## 13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

*Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes. Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.*

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

13.9 Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed

time in the microplate reader prepared with the following settings:

| Mode | Kinetic |
|---|---|
| Wavelength: | 600 nm |
| Time: | up to 20 min |
| Interval: | 20 sec - 1 min |
| Shaking: | Shake between readings |

Δ **Note**: that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

13.10 Analyze the data as described below.

# 14. Calculations

**14.1** Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

**14.2** **Create a standard curve** by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

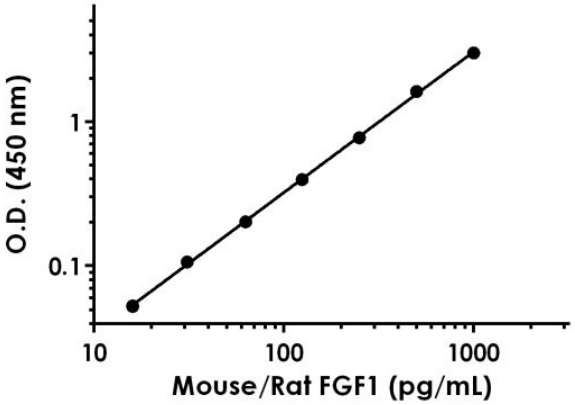
Δ **Note:** Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

**14.3** Determine the concentration of the target protein in the sample by interpolating the blank control subtracted **absorbance values against the standard curve**. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

**14.4** Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

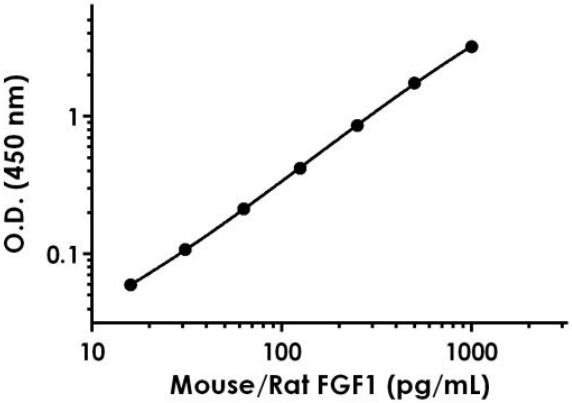
## 15. Typical Data

**Typical standard curve** – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



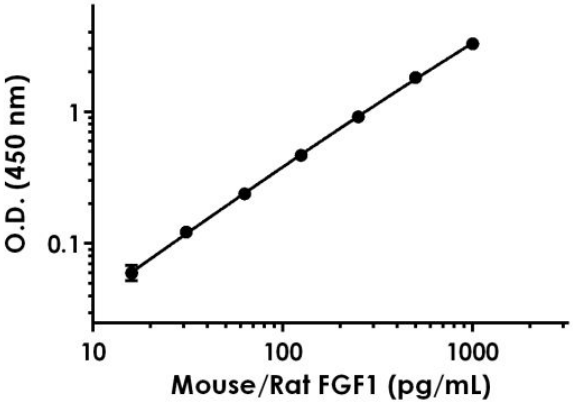

| Standard Curve Measurements | | | |
|---|---|---|---|
| Concentration (pg/mL) | O.D 450 nm | | Mean O.D |
| | 1 | 2 | |
| 0 | 0.046 | 0.049 | 0.048 |
| 16 | 0.1 | 0.101 | 0.1 |
| 31 | 0.156 | 0.152 | 0.154 |
| 63 | 0.253 | 0.245 | 0.249 |
| 125 | 0.443 | 0.444 | 0.443 |
| 250 | 0.795 | 0.849 | 0.822 |
| 500 | 1.632 | 1.697 | 1.664 |
| 1,000 | 2.979 | 3.113 | 3.046 |

**Figure 1**. Example of mouse/rat FGF1 standard curve in 1X Cell Extraction Buffer PTR. The FGF1 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.




| Standard Curve Measurements | | | |
|---|---|---|---|
| Concentration (pg/mL) | O.D 450 nm | | Mean O.D |
| | 1 | 2 | |
| 0 | 0.048 | 0.048 | 0.048 |
| 16 | 0.108 | 0.107 | 0.107 |
| 31 | 0.156 | 0.155 | 0.155 |
| 63 | 0.26 | 0.26 | 0.26 |
| 125 | 0.467 | 0.468 | 0.467 |
| 250 | 0.885 | 0.925 | 0.905 |
| 500 | 1.759 | 1.829 | 1.794 |
| 1,000 | 3.241 | 3.275 | 3.258 |

**Figure 2**. Example of mouse/rat FGF1 standard curve in Sample Diluent NS. The FGF1 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.




| Standard Curve Measurements | | | |
|---|---|---|---|
| Concentration (pg/mL) | O.D 450 nm | | Mean O.D |
| | 1 | 2 | |
| 0 | 0.094 | 0.096 | 0.095 |
| 16 | 0.149 | 0.161 | 0.155 |
| 31 | 0.222 | 0.212 | 0.217 |
| 63 | 0.329 | 0.338 | 0.334 |
| 125 | 0.561 | 0.561 | 0.561 |
| 250 | 0.959 | 1.058 | 1.009 |
| 500 | 1.888 | 1.941 | 1.915 |
| 1,000 | 3.328 | 3.397 | 3.362 |

**Figure 3**. Example of mouse/rat FGF1 standard curve in Sample Diluent 25BS. The FGF1 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

# 16. Typical Sample Values

## SENSITIVITY –

The Minimal Detectable Dose was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

| Sample Diluent Buffer | n= | Minimal Detectable Dose |
|---|---|---|
| 1X Cell Extraction Buffer PTR | 27 | 16 pg/mL |
| Sample Diluent NS | 26 | 2 pg/mL |
| Sample Diluent 25BS | 26 | 8 pg/mL |

## RECOVERY –

Three concentrations of mouse/rat FGF1 were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

| Sample Type | Average % Recovery | Range (%) |
|---|---|---|
| Mouse Brain Lysate (1.9 µg/ml) | 118 | 105-126 |
| Rat Brain Lysate (3.9 µg/ml) | 116 | 107-130 |
| Mouse Urine (6.5%) | 111 | 95-135 |
| Rat Urine (25%) | 107 | 90-122 |
| Mouse Plasma-Citrate (1.6%) | 119 | 111-125 |
| Mouse Plasma-EDTA (1.6%) | 105 | 102-109 |
| Mouse Serum (1.6%) | 116 | 111-119 |
| Rat Plasma-Citrate (12.5%) | 109 | 104-114 |
| Rat Plasma-EDTA (25%) | 104 | 100-112 |
| Rat Serum (12.5%) | 103 | 101-104 |
| RAW 264.7 Supernatant (1.6%) | 117 | 108-125 |

## Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native FGF1 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent PTR.

| Dilution Factor | Interpolated value | 31 µg/ml Mouse Brain Tissue Extract | 63 µg/ml Rat Brain Tissue Extract |
|---|---|---|---|
| Undiluted | pg/mL | 711 | 786 |
| | % Expected | 100 | 100 |
| 2 | pg/mL | 371 | 424 |
| | % Expected | 104 | 108 |
| 4 | pg/mL | 184 | 210 |
| | % Expected | 104 | 107 |
| 8 | pg/mL | 85 | 100 |
| | % Expected | 96 | 102 |
| 16 | pg/mL | 41 | 47 |
| | % Expected | 92 | 96 |

Recombinant FGF1 was spiked into the following biological samples and diluted in a 2-fold dilution series. Serum, plasma and supernatant samples were diluted in Sample Diluent NS. Urine samples were diluted in Sample Diluent 25BS.

| Dilution Factor | Interpolated value | 50% Mouse Serum | 25% Mouse Plasma (Citrate) | 50% Mouse Plasma (EDTA) | 25% Mouse Urine | 95% RAW 264.7 Supernatant |
|---|---|---|---|---|---|---|
| Undiluted | pg/mL | 1102 | 1281 | 1120 | 513 | 584 |
| | % Expected | 100 | 100 | 100 | 100 | 100 |
| 2 | pg/mL | 606 | 644 | 589 | 281 | 288 |
| | % Expected | 110 | 101 | 105 | 110 | 99 |
| 4 | pg/mL | 312 | 314 | 298 | 133 | 139 |
| | % Expected | 113 | 98 | 106 | 104 | 96 |
| 8 | pg/mL | 152 | 153 | 146 | 61 | 69 |
| | % Expected | 110 | 95 | 104 | 96 | 95 |
| 16 | pg/mL | 76 | 71 | 75 | NL | 34 |
| | % Expected | 110 | 89 | 107 | NL | 92 |

NL – Non-Linear

| Dilution Factor | Interpolated value | 25% Rat Serum | 25% Rat Plasma (Citrate) | 50% Rat Plasma (EDTA) | 50% Rat Urine |
|---|---|---|---|---|---|
| Undiluted | pg/mL | 433 | 500 | 497 | 551 |
| | % Expected value | 100 | 100 | 100 | 100 |
| 2 | pg/mL | 239 | 266 | 24 | 286 |
| | % Expected value | 110 | 106 | 99 | 104 |
| 4 | pg/mL | 121 | 130 | 128 | 141 |
| | % Expected value | 112 | 104 | 103 | 102 |
| 8 | pg/mL | 62 | 63 | 65 | 69 |
| | % Expected value | 115 | 101 | 104 | 101 |
| 16 | pg/mL | 34 | 34 | 32 | NL |
| | % Expected value | 126 | 108 | 102 | NL |

NL – Non-Linear

PRECISION –

Mean coefficient of variations of interpolated values from three concentrations of mouse brain tissue extract within the working range of the assay.

| | Intra-Assay | Inter-Assay |
|---|---|---|
| n = | 5 | 3 |
| CV(%) | 2.7 | 3.7 |

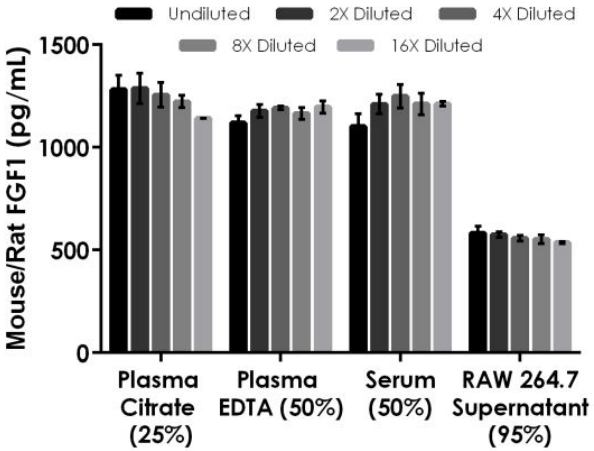



Figure 4. Interpolated concentrations of spiked mouse/rat FGF1 in mouse serum, plasma and cell culture supernatant samples. The concentrations of FGF1 were measured in duplicates, interpolated from the FGF1 standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 50%, plasma (citrate) 25%, plasma (EDTA) 50%, and RAW 264.7 supernatant 95%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2).

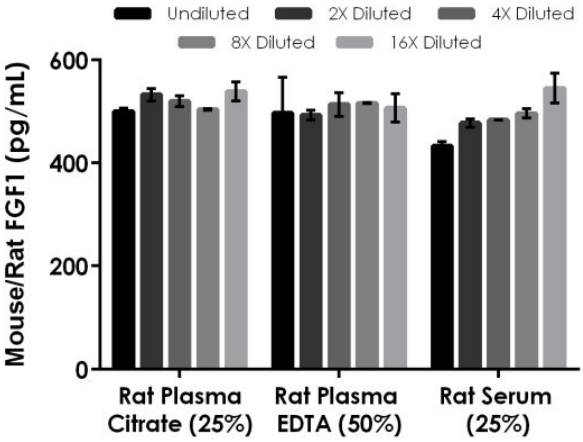



**Figure 5.** Interpolated concentrations of spiked mouse/rat FGF1 in rat serum and plasma samples. The concentrations of FGF1 were measured in duplicates, interpolated from the FGF1 standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 25%, plasma (citrate) 25% and plasma (EDTA) 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2).

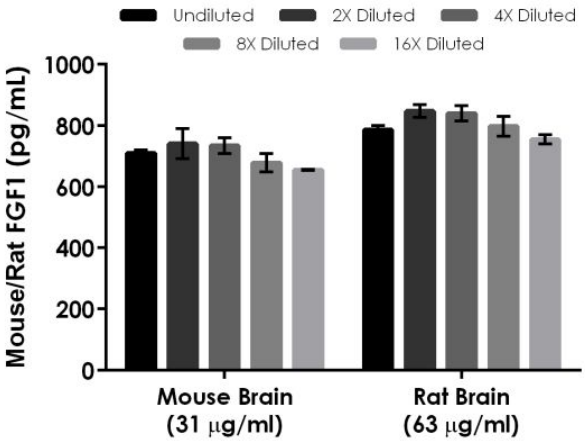



Figure 6. Interpolated concentrations of native mouse/rat FGF1 in mouse and rat brain tissue extract based on a 31 or 63 µg/mL extract load, respectively. The concentrations of FGF1 were measured in duplicate and interpolated from the FGF standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean FGF1 concentration was determined to be 697 pg/mL in mouse brain tissue extract and 796 pg/mL in rat brain tissue extract.

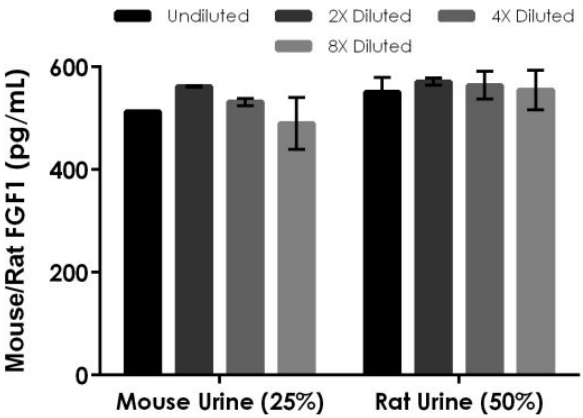



Figure 7. Interpolated concentrations of spiked FGF1 in mouse and rat urine. The concentrations of FGF1 were measured in duplicate and interpolated from the FGF1 standard curve and corrected for sample dilution. Undiluted samples are as follows: mouse urine 25%, rat urine 50%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2).

# 17. Assay Specificity

This kit recognizes both native and recombinant mouse/rat FGF1 protein in serum, plasma (citrate and EDTA), urine, cell culture supernatant, and cell and tissue extract samples only.

Plasma (heparin) samples are not compatible with this kit.

Milk samples have not been tested with this kit.

## INTERFERENCE

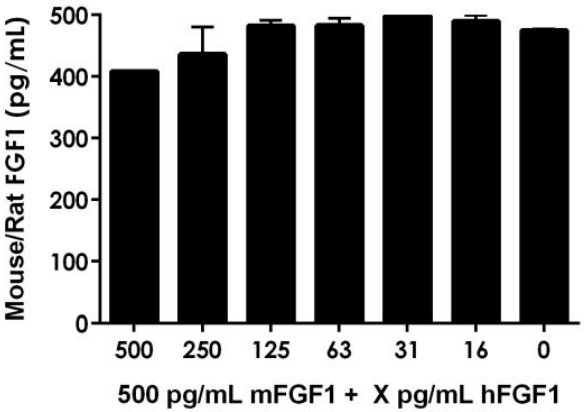



**Figure 8.** Human FGF1 active protein (hFGF1) was added at the indicated concentrations to 500 pg/ml mouse FGF1 (mFGF1) to test for Interference. Only 14% interference was observed with the highest dose of hFGF1.

# 18. Troubleshooting

| Problem | Reason | Solution |
|---|---|---|
| **Difficulty pipetting lysate; viscous lysate.** | Genomic DNA solubilized | Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction. |
| **Poor standard curve** | Inaccurate Pipetting | Check pipettes |
| | Improper standard dilution | Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing |
| **Low Signal** | Incubation times too brief | Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation |
| | Inadequate reagent volumes or improper dilution | Check pipettes and ensure correct preparation |
| | Incubation times with TMB too brief | Ensure sufficient incubation time until blue color develops prior addition of Stop solution |
| **Large CV** | Plate is insufficiently washed | Review manual for proper wash technique. If using a plate washer, check all ports for obstructions. |
| | Contaminated wash buffer | Prepare fresh wash buffer |
| **Low sensitivity** | Improper storage of the ELISA kit | Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB Development Solution solution protected from light. |
| **Precipitate in Diluent** | Precipitation and/or coagulation of components within the Diluent. | Precipitate can be removed by gently warming the Diluent to 37°C. |

# 19. Notes

## Technical Support



### For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)