# Human/Mouse/Rat GDF-8 Sandwich ELISA Kit Datasheet

Please read it entirely before use

**Catalogue Number:** KE00120

**Size:** 5*96T

**Sensitivity:** 2.3 pg/mL

**Range:** 15.6-1000 pg/mL, 31.25-2000 pg/mL

**Usage:** For the quantitative detection of human/mouse/rat GDF-8 concentrations in serum, plasma, cell culture supernatant and tissue lysate.

**This product is for research use only and not for use in human or animal therapeutic or diagnostic.**



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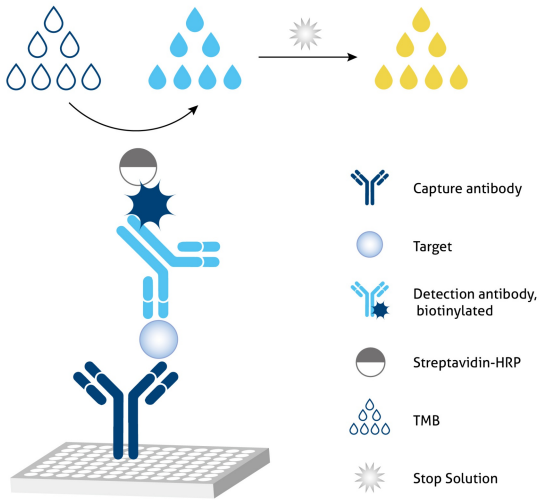
## 1. Background

Growth Differentiation Factor 8 (GDF-8), also called myostatin, is a member of the transforming growth factor (TGF)-β super-family. GDF-8 is specifically expressed during embryonic development and in adult skeletal muscle, functioning as a negative regulatory protein. GDF-8 is also expressed in the human reproductive system, such as in granulosa cells, follicular fluid and trophoblasts. In addition, GDF-8 has also functions in heart and adipose tissue, and is related to interstitial fibrosis in the heart, human cancer cachexia, and activation of inflammatory cytokines and insulin resistance.

## 2. Principle




**Sandwich ELISA structure (Detection antibody labeled with biotin)**

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with biotin also binds to the analyte. Streptavidin-HRP binds to the biotin. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

## 3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.



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## 4. Kit Components and Storage

| | | |
|---|---|---|
| **Microplate** - antibody coated 96 - well microplate (8 well × 12 strips) | 5 plates | **Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.** |
| **Protein standard** - 2000 pg/bottle; lyophilized | 10 bottles | |
| **Detection antibody, biotinylated (100×)** - 600 μL/vial* | 1 vial | |
| **Streptavidin-horseradish peroxidase (HRP) (100×)** - 600 μL/vial* | 1 vial | |
| **Sample Diluent PT 3-ef** - 150 mL/bottle. For human serum and plasma | 1 bottle | |
| **Sample Diluent PT 4-ef** - 150 mL/bottle. For mouse/rat serum, plasma and serum-free cell culture supernatant | 1 bottle | |
| **Sample Diluent PT 5-ef** - 150 mL/bottle. For tissue lysate | 1 bottle | |
| **Detection Diluent** - 150 mL/bottle | 1 bottle | |
| **Wash Buffer Concentrate (20×)** - 150 mL/bottle | 1 bottle | |
| **Extraction Reagent** - 150 mL/bottle | 1 bottle | |
| **Tetramethylbenzidine Substrate (TMB)** - 60 mL/bottle | 1 bottle | |
| **Stop Solution** - 60 mL/bottle | 1 bottle | |
| **Plate Cover Seals** | 15 pieces | |

* Centrifugation immediately before use

## 5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision



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## 6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20℃. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20℃. Avoid repeated freeze-thaw cycles.

6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

6.4 Tissue Lysate:

1) Rinse tissue with PBS, cut into 1-2 mm pieces.

2) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing tissue lysis.

3) Add 1 mL of Extraction Reagent containing protease inhibitor cocktail per 100 mg tissue.

4) Homogenize the tissue completely using desired method on ice, Incubate on ice for 30 minutes, use ultrasound to break up the cells.

5) Centrifuge tissue homogenates at 10,000 x g for 5 minutes at 4℃. Collect the supernatant, assay immediately or aliquot and store at -20℃.

6) Measure the concentration of total protein in tissue homogenates using BCA assay.

7) Avoid protein degradation by performing all the above procedures on ice where possible.



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## 7. Regent Preparation

**7.1 Wash Buffer (1X):** If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

**7.2 Detection Antibody (1X):** Dilute 100X Detection Antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 μL 100X Detection Antibody + 990 μL Detection Diluent (Centrifuge the 100 X Detection Antibody solution for a few seconds prior to use).

**7.3 Streptavidin-HRP (1X):** Dilute 100X Streptavidin-HRP 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 μL 100X Streptavidin-HRP + 990 μL Detection Diluent (Centrifuge the 100X Streptavidin-HRP solution for a few seconds prior to use).

**7.4 Sample Dilution:** Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:4 is recommended for serum-free cell culture supernatant; 11:16 is recommended for human serum and plasma;1:40 is recommended for mouse serum; 1:40 is recommended for rat serum; :8 is recommended for tissue lysate.

To remove the pro-peptide from GDF-8, prepare the following solutions for acid activation and neutralization. The solutions may be stored in polypropylene bottles at room temperature for up to one month.

1 N HCl (100 mL) - To 91.67 mL of deionized water, slowly add 8.33 mL of 12 N HCl. Mix well.

1.2 N NaOH/0.5 M HEPES (100 mL) - To 75 mL of deionized water, slowly add 12 mL of 10 N NaOH. Mix well. Add 11.9 g of HEPES. Mix well. Bring final volume to 100 mL with deionized water.

For each new lot of acidifcation and neutralization reagents, measure the pH of several representative samples after neutralization to ensure that it is within pH 7.2-7.6. Adjust the volume and corresponding dilution factor of the neutralization reagent as need.

Note：Do not activate the kit standards. The kit standards contain active recombinant GDF-8.

Use the chart below for volumes of 1N HCL, 1.2N NaOH/0.5 M HEPES, and sample diluent used for specific sample types.

1、Add 1N HCL to sample. Mix well. Incubate for 10 minutes at room temperature

2、Add 1.2N NaOH/0.5 M HEPES. Mix well.

3、Add sample diluent. Mix well and assay within 2 hours.

| Sample Type | Sample (uL) | 1N HCL(uL) | 1.2N NaOH(uL) | sample diluent | Final Dilution Factor |
|---|---|---|---|---|---|
| Serum-free cell culture supernatant | 100 | 50 | 50 | 200 | 1:4 |
| Human serum & plasma | 20 | 10 | 10 | 280 | 1:16 |
| Mouse serum & plasma | 20 | 10 | 10 | 760 | 1:40 |
| Rat serum & plasma | 20 | 10 | 10 | 760 | 1:40 |
| Tissue lysate | 100 | 50 | 50 | 600 | 1:8 |



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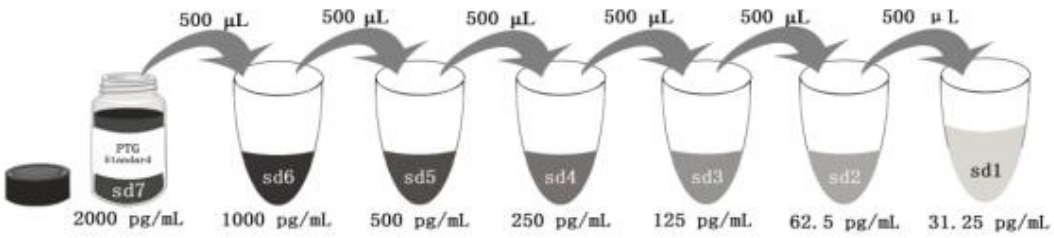
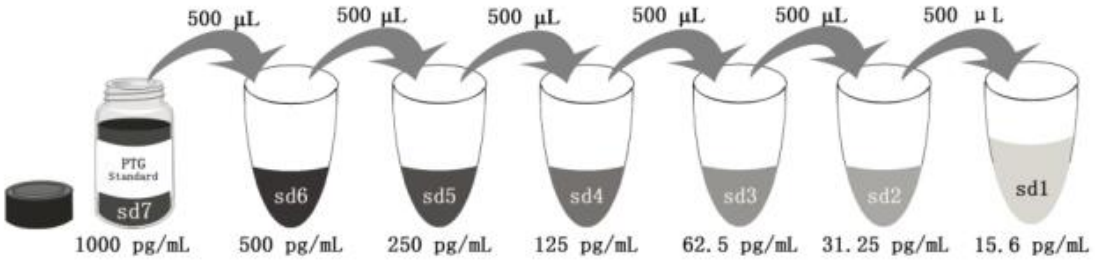
### 7.5 Standard Serial Dilution:

For huamn serum and plasma, add 1 mL Sample Diluent PT 3-ef in protein standard; For mouse or rat serum, plasma and serum-free cell culture supernatant, add 1 mL Sample Diluent PT 4-ef in protein standard.




| Add # µL of Standard diluted in the previous step | — | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
|---|---|---|---|---|---|---|---|
| # µL of Sample Diluent **PT 3-ef or PT 4-ef** | **1000 µL** | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| | "sd7" | "sd6" | "sd5" | "sd4" | "sd3" | "sd2" | "sd1" |

For tissue lysate, add 2 mL Sample Diluent PT 5-ef in protein standard.




| Add # µL of Standard diluted in the previous step | — | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
|---|---|---|---|---|---|---|---|
| # µL of Sample Diluent **PT 5-ef** | **2000 µL** | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| | "sd7" | "sd6" | "sd5" | "sd4" | "sd3" | "sd2" | "sd1" |



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## 8. Assay Procedure Summary

**Bring all reagents to room temperature before use (Detection antibody and Streptavidin-HRP can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.**

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group,standard group and sample group, add 100 μL of each standard and sample to the appropriate wells.(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 μL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 μL of 1X Detection Antibody solution (refer to Reagent Preparation7.2) to each well. Seal plate with cover seal and incubate for 1 hour at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Add 100 μL of 1X Streptavidin-HRP solution (refer to Reagent Preparation7.3) to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37°C .

8.8 Repeat wash step in 8.4.

8.9 Signal development: Add 100 μL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.10 Quenching color development: Add 100 μL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.11 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.12 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve- fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

| Step | Reagent | Volume | Incubation | Wash | Notes |
|---|---|---|---|---|---|
| 1 | Standard and Samples | 100 µL | 120 min | 4 times | Cover Wells incubate at 37°C |
| 2 | Diluent Antibody Solution | 100 µL | 60 min | 4 times | Cover Wells incubate at 37°C |
| 3 | Diluent HRP Solution | 100 µL | 40 min | 4 times | Cover Wells incubate at 37°C |
| 4 | TMB Substrate | 100 µL | 15-20 min | Do not wash | Incubate in the dark at 37°C |
| 5 | Stop Solution | 100 µL | 0 min | Do not wash | - |
| 6 | Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes. | | | | |



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# 9. Validation Data

## 9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



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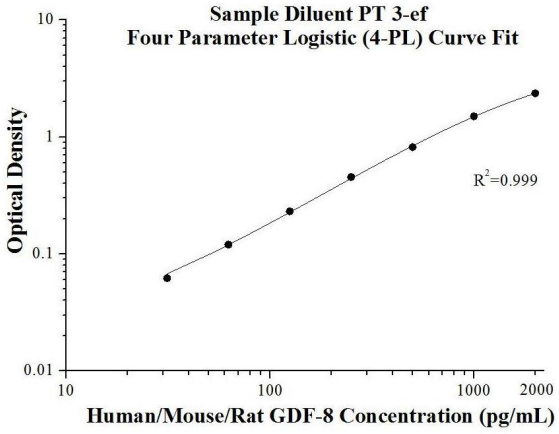
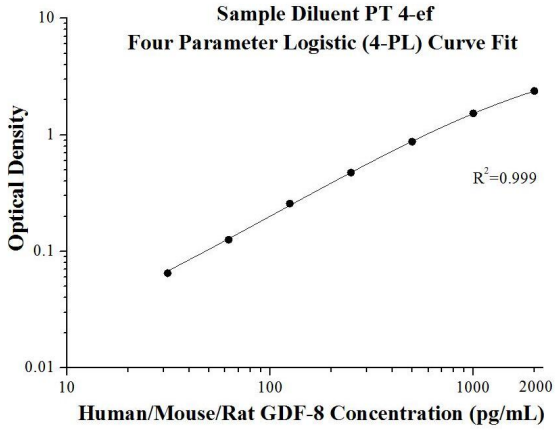
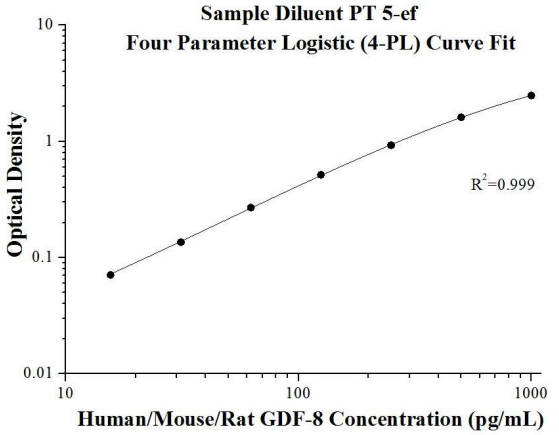



| (pg/mL) | O.D | Average | Corrected |
|---|---|---|---|
| 0 | 0.043<br>0.041 | 0.042 | - |
| 31.25 | 0.107<br>0.101 | 0.104 | 0.062 |
| 62.5 | 0.159<br>0.165 | 0.162 | 0.120 |
| 125 | 0.28<br>0.266 | 0.273 | 0.231 |
| 250 | 0.506<br>0.186 | 0.496 | 0.454 |
| 500 | 0.845<br>0.872 | 0.859 | 0.817 |
| 1000 | 1.561<br>1.519 | 1.540 | 1.498 |
| 2000 | 2.398<br>2.394 | 2.396 | 2.354 |




| (pg/mL) | O.D | Average | Corrected |
|---|---|---|---|
| 0 | 0.058<br>0.054 | 0.056 | - |
| 31.25 | 0.12<br>0.122 | 0.121 | 0.065 |
| 62.5 | 0.188<br>0.175 | 0.182 | 0.126 |
| 125 | 0.316<br>0.31 | 0.313 | 0.257 |
| 250 | 0.55<br>0.509 | 0.530 | 0.474 |
| 500 | 0.952<br>0.906 | 0.929 | 0.873 |
| 1000 | 1.616<br>1.547 | 1.582 | 1.526 |
| 2000 | 2.431<br>2.427 | 2.429 | 2.373 |




| (pg/mL) | O.D | Average | Corrected |
|---|---|---|---|
| 0 | 0.071<br>0.07 | 0.071 | - |
| 15.6 | 0.144<br>0.139 | 0.142 | 0.071 |
| 32.25 | 0.203<br>0.21 | 0.207 | 0.136 |
| 62.5 | 0.34<br>0.34 | 0.340 | 0.269 |
| 125 | 0.586<br>0.585 | 0.58 | 0.515 |
| 250 | 0.995<br>0.996 | 0.996 | 0.925 |
| 500 | 1.696<br>1.666 | 1.681 | 1.610 |
| 1000 | 2.553<br>2.546 | 2.550 | 2.479 |



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## 9.2 Precision

**Intra-assay Precision** (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

| Intra-assay Precision | | | | |
|---|---|---|---|---|
| Sample | n | Mean (pg/mL) | SD | CV% |
| 1 | 20 | 52.0 | 1.5 | 2.8 |
| 2 | 20 | 212.4 | 7.2 | 3.4 |
| 3 | 20 | 877.1 | 28.3 | 3.2 |

| Inter-assay Precision | | | | |
|---|---|---|---|---|
| Sample | n | Mean (pg/mL) | SD | CV% |
| 1 | 24 | 54.1 | 3.0 | 5.5 |
| 2 | 24 | 217.9 | 6.6 | 3.0 |
| 3 | 24 | 854.3 | 27.4 | 3.2 |

## 9.3 Recovery

The recovery of human/mouse/rat GDF-8 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

| Sample Type | | Average% of Expected | Range (%) |
|---|---|---|---|
| Human plasma | 1:32 | 110 | 90-121 |
| | 1:64 | 111 | 101-123 |
| Mouse serum | 1:160 | 114 | 107-121 |
| | 1:320 | 111 | 107-115 |
| Rat serum | 1:40 | 86 | 75-110 |
| | 1:80 | 103 | 87-117 |
| Serum-free cell culture supernatant | 1:4 | 83 | 73-91 |
| | 1:8 | 92 | 71-109 |
| Tissue lysate | 1:8 | 97 | 82-111 |
| | 1:16 | 89 | 81-101 |



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## 9.4 Sample values

| Sample Type | Mean (pg/mL) | Range (pg/mL) |
|---|---|---|
| Human plasma (n=16) | 5,904 | 3,964-8,856 |
| Mouse serum (n=16) | 59,633 | 41,505-77,261 |
| Rat serum (n=16) | 7,530 | 4,225-10,262 |

**Tissue lysate** -Dissect the tissue of interest and wash briefly with chilled **1X PBS** to remove any blood if necessary, cut the tissue into smaller pieces whilst keeping it on ice. Transfer the tissue to a homogenizer and add **Extraction Reagent** with protease inhibitor. In general, add 500 μL **Extraction Reagent** for approximately every 10 mg of tissue. Homogenize thoroughly and keep the sample on ice for 30 min. Sonicate the sample and centrifuge at 10,000 x g, then transfer the supernatant to assay.

| Tissue Type | GDF-8 (pg/mL) | Total protein (mg/mL) |
|---|---|---|
| Mouse skelectal muscle | 1,464 | 8.2 |

***1X PBS** For 1000 mL

10 mM $Na_2HPO4$, 1.8 mM $NaH_2PO4$, 140 mM NaCl. Adjust pH to 7.4 and add $ddH_2O$ to 1000 mL.

## 9.5 Sensitivity

The minimum detectable dose of human/mouse/rat GDF-8 is 2.3 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.



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## 9.6 Linearity

To assess the linearity of the assay, Serum-free cell culture supernatant samples were spiked with high concentrations of human/mouse/rat GDF-8 in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Serum and tissue lysate were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

| Sample Type | | Average% of Expected | Range (%) |
|---|---|---|---|
| Human serum (Sample Diuent PT3-ef) | 1:8 | 89 | 84-100 |
| | 1:16 | 104 | 100-115 |
| | 1:32 | 106 | 101-113 |
| | 1:64 | 103 | 97-116 |
| Mouse serum (Sample Diuent PT4-ef) | 1:40 | 99 | 85-119 |
| | 1:80 | 98 | 92-100 |
| | 1:160 | 107 | 100-112 |
| | 1:320 | 105 | 84-121 |
| Rat serum (Sample Diuent PT4-ef) | 1:40 | 90 | 82-101 |
| | 1:80 | 100 | 99-101 |
| | 1:160 | 108 | 93-126 |
| | 1:320 | 95 | 88-103 |
| Serum-free cell culture supernatant (Sample Diuent PT4-ef) | 1:4 | 74 | 73-74 |
| | 1:8 | 95 | 85-105 |
| | 1:16 | 95 | 87-104 |
| | 1:32 | 100 | 89-112 |
| Tissue lysate (Sample Diuent PT5-ef) | 1:8 | 78 | 77-79 |
| | 1:16 | 100 | 99-101 |
| | 1:32 | 115 | 114-115 |

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