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

For the quantitative detection of Human/Mouse/Rat GDF-8 concentrations in serum, plasma, cell culture supernatants and tissue lysates.

General Information

Catalogue Number	KE00120
Product Name	Human/Mouse/Rat GDF-8 Sandwich ELISA Kit
Species cross-reactivity	Human/Mouse/Rat
Range (calibration Range)	15.6-1000 pg/mL, 31.25-2000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	2660(Human)/17700(Mouse)/29152(Rat)
SwissProt	O14793(Human)/O08689(Mouse)/O35312(Rat)

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Kit Components & Storage

Microplate - antibody coated 96 - well microplate (8 well × 12 strips)	1 plate	**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*	2 bottles	
Detection antibody, biotinylated (100X) - 120 μL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 μL/vial	1 vial	
Sample Diluent PT 3-ef - 30 mL/bottle. For human serum and plasma.	1 bottle	
Sample Diluent PT 4-ef - 30 mL/bottle. For mouse/rat serum, plasma and serum-free cell culture supernatants.	1 bottle	
Sample Diluent PT 5-ef - 30 mL/bottle. For tissue lysates.	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Extraction Reagent - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

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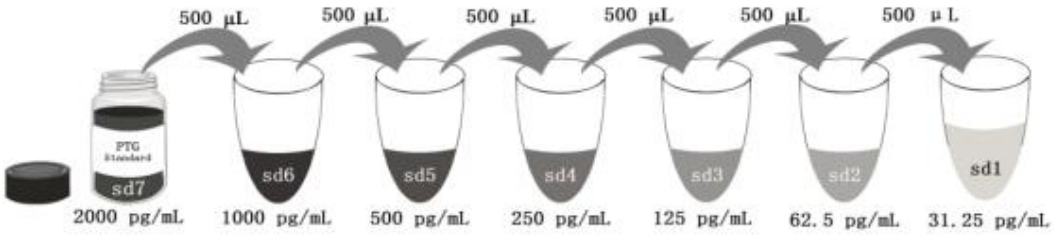
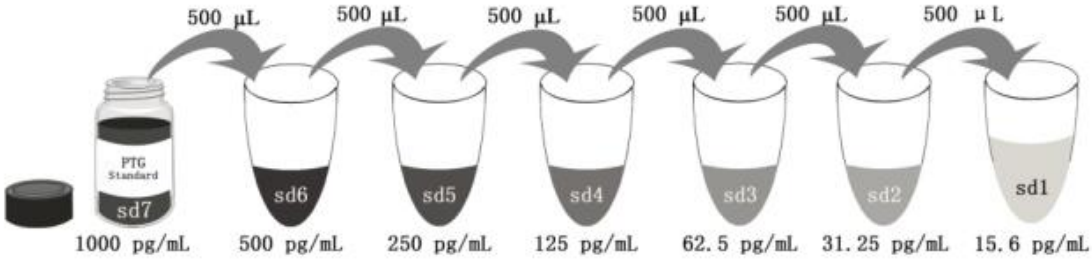
NB: Do not use the kit after the expiration date.
Sample Diluent PT 3-ef is for protein standard, human serum and plasma.
Sample Diluent PT 4-ef is for protein standard, mouse/rat serum, plasma and cell culture supernatants.
Sample Diluent PT 5-ef is for protein standard and tissue lysates.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **1 mL** Sample Diluent PT 3-ef or PT 4-ef in protein standard. This reconstitution gives a stock solution of 2000 pg/mL.
*Add **2 mL** Sample Diluent PT 5-ef in protein standard. This reconstitution gives a stock solution of 1000 pg/mL.



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"



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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Product Description

KE00120 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The GDF-8 ELISA kit is to be used to detect and quantify protein levels of endogenous GDF-8. The assay recognizes human,mouse and Rat GDF-8. An antibody specific for GDF-8 has been pre-coated onto the microwells. The GDF-8 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for GDF-8 is added to detect the captured GDF-8 protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

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.

Sample Preparation

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- To 88 mL of deionized water, slowly add 12 mL of 10 N NaOH. Mix well.
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, 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(uL). Mix well.
3、Add sample diluent. Mix well and assay within 2 hours.

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Sample Type	Sample (uL)	1N HCL(uL)	1.2N NaOH(uL)	sample diluent	Final Dilution Factor
Serum-free cell culture supernatans	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 lysates	100	50	50	600	1:8

Safety Notes

This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

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.				

Example data

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

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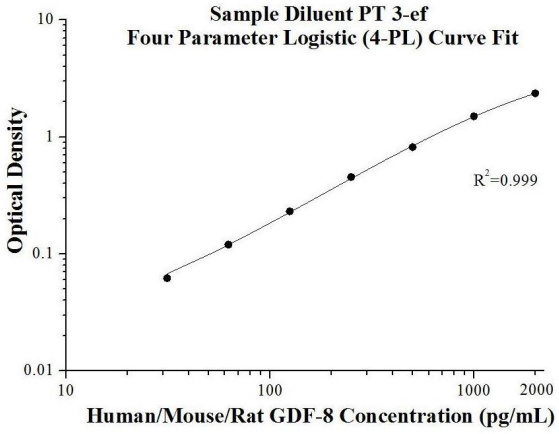
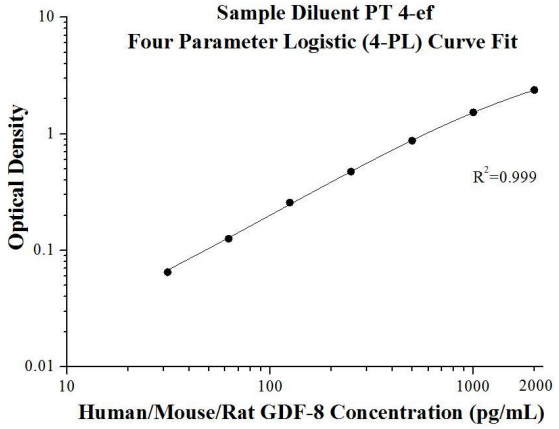
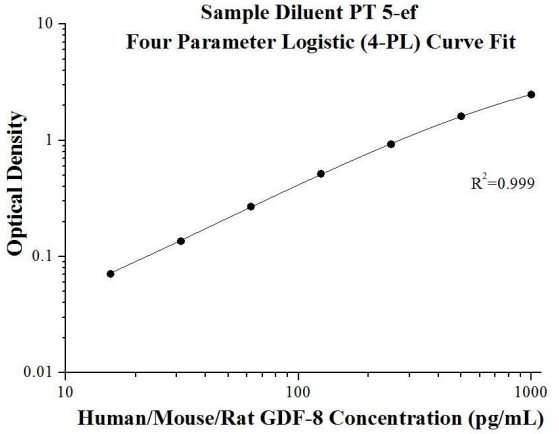


(pg/mL)	O.D	Average	Corrected
0	0.043 0.041	0.042	-
31.25	0.107 0.101	0.104	0.062
62.5	0.159 0.165	0.162	0.120
125	0.28 0.266	0.273	0.231
250	0.506 0.186	0.496	0.454
500	0.845 0.872	0.859	0.817
1000	1.561 1.519	1.540	1.498
2000	2.398 2.394	2.396	2.354



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



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

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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

Recovery

The recovery of GDF-8 spiked to three different levels in four samples 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 supernatants	1:4	83	73-91
	1:8	92	71-109
Tissue lysates	1:8	97	82-111
	1:16	89	81-101

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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 lysates -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.

Sensitivity

The minimum detectable dose of 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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Linearity

To assess the linearity of the assay, Serum-free cell culture supernatants samples were spiked with high concentrations of 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 lysates 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 supernatants (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 lysates (Sample Diuent PT5-ef)	1:8	78	77-79
	1:16	100	99-101
	1:32	115	114-115

References

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