

# Quantitation of Myostatin/GDF-8 in Human Serum Using an Enzyme Linked Immunosorbent Assay (ELISA)

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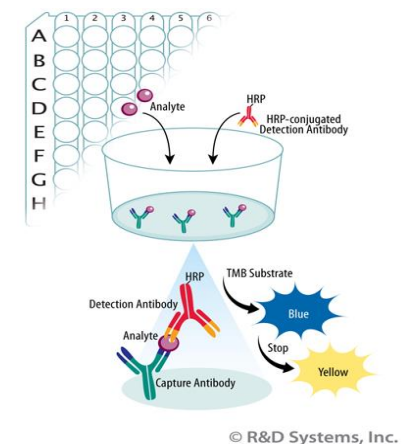


## INTRODUCTION

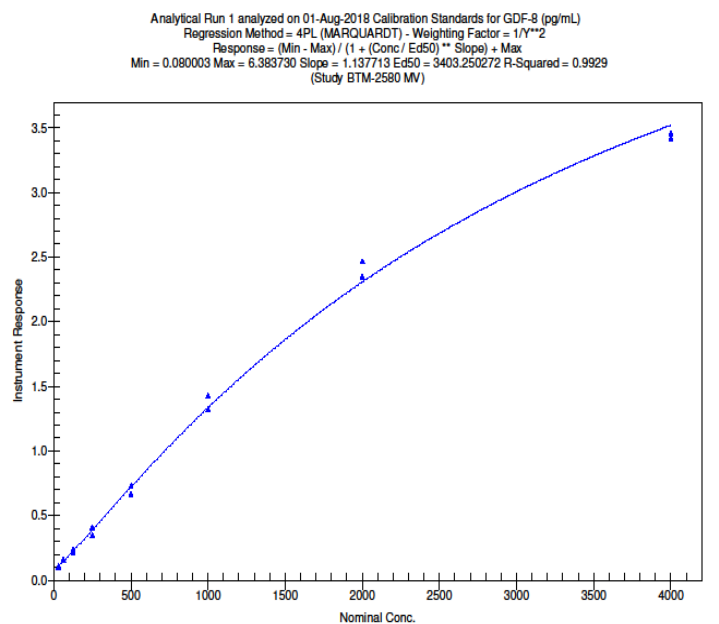
Growth Differentiation Factor 8 (GDF-8), also known as Myostatin, is a secreted TGF-β superfamily protein that is expressed in developing and adult skeletal muscle. It controls myoblast proliferation and is a potent negative regulator of skeletal muscle mass. The amino acid sequence of mature GDF-8 is conserved between human, mouse, and rat. The study aimed to develop and validate a sensitive immunoassay for the quantitation of GDF8 in human serum as a potential cardiac biomarker for clinical studies in heart failure. The Quantikine® GDF-8/Myostatin Immunoassay is a solid phase ELISA assay designed to measure GDF-8 levels in serum and plasma. This assay employs the quantitative sandwich enzyme immunoassay technique. The assay parameters, including precision and accuracy, reproducibility and detectability, parallelism/dilution of linearity and stability was assessed during assay validation. The assay sensitivity is 31.25 pg/mL with the MRD of 1:8.

## METHOD

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mature GDF-8 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells, and any GDF-8 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for mature GDF-8 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of GDF-8 bound in the initial step. The color development is stopped and the intensity of the color is measured.



## ONE REPRESENTATIVE STANDARD CURVE



## TWO REPRESENTATIVE STANDARD DATA

Run ID	Run Details	Std 8	Std 7	Std 6	Std 5	Std 4	Std 3	Std 2	Std 1
		31.25 pg/mL	62.50 pg/mL	125.00 pg/mL	250.00 pg/mL	500.00 pg/mL	1,000.00 pg/mL	2,000.00 pg/mL	4,000.00 pg/mL
Run 1	#1	31.25	62.50	125.00	250.00	500.00	1,000.00	2,000.00	4,000.00
	#2	29.32	73.03	138.30	265.21	457.64	1081.47	2201.93	3856.80
	Intra run Mean	27.04	71.91	129.87	242.04	482.59	1035.45	2126.95	3814.19
	Intra run SD	3.22	1.58	11.93	32.77	35.28	65.09	106.04	60.27
	Intra run %CV	11.9	2.2	9.2	13.5	7.3	6.3	5.0	1.6
	Intra run %RE	-13.5	15.1	3.9	-3.2	-3.5	3.5	6.3	-4.6
Run 2	n	2	2	2	2	2	2	2	2
	#1	32.19	66.09	135.38	266.58	525.16	1045.34	2294.35	3728.34
	#2	25.80	64.08	128.80	228.91	448.56	914.44	2368.85	3644.48
	Intra run Mean	29.00	65.09	132.09	247.75	486.86	979.89	2331.60	3686.41
	Intra run SD	4.52	1.42	4.65	26.64	54.16	92.56	52.68	59.30
	Intra run %CV	15.6	2.2	3.5	10.8	11.1	9.4	2.3	1.6
Run 2	Intra run %RE	-7.2	4.1	5.7	-0.9	-2.6	-2.0	16.6	-7.8
	n	2	2	2	2	2	2	2	2

## GDF-8 REPRODUCIBILITY IN HUMAN SERUM

Serum Samples	Initial Value			Re-assay Value			%Difference
	Lot #	Mean (pg/mL)	%CV	Serum Samples	Mean (pg/mL)	%CV	
HS01	BRH1503831	4336.53	1.3	HS01	4315.92	2.0	-0.5
HS02	BRH1503833	4339.18	0.5	HS02	4208.83	2.8	-3.0
HS03	BRH1503834	14838.11	0.8	HS03	13850.34	1.7	-6.9
HS04	BRH1503836	15539.66	1.0	HS04	14253.27	2.0	-8.6
HS05	BRH1503837	8919.09	2.8	HS05	8629.70	3.9	-3.3
HS06	BRH1503838	5002.28	0.8	HS06	5045.62	3.0	0.9
HS07	BRH1503839	4911.02	2.2	HS07	4947.25	1.4	0.7
HS08	BRH1503844	2772.98	1.9	HS08	2685.42	2.5	-3.2
HS09	BRH1503848	2360.25	0.6	HS09	2348.66	0.6	-0.5
HS10	BRH1503850	2038.12	5.5	HS10	2000.65	2.2	-1.9

## GDF-8 PARALLELISM IN HUMAN SERUM

Serum Samples	Lot #	1X			4X			8X		16X			32X		
		Mean (pg/mL)	%CV	%RE	Mean (pg/mL)	%CV	%RE	Mean (pg/mL)	%CV	Mean (pg/mL)	%CV	%RE	Mean (pg/mL)	%CV	%RE
HS01	BRH1503831	890.51	0.2	-79.5	4284.10	0.7	-1.2	4336.53	1.3	4502.44	1.1	3.8	4990.87	2.1	15.1
HS02	BRH1503833	905.57	2.1	-79.1	4419.17	0.7	1.8	4339.18	0.5	4478.54	0.4	3.2	4802.52	0.2	10.7
HS03	BRH1503834	3469.47	1.2	-76.6	14397.60	1.1	-3.0	14838.11	0.8	14151.57	0.4	-4.6	14458.04	0.2	-2.6
HS04	BRH1503836	3118.75	0.8	-79.9	14675.48	1.2	-5.6	15539.66	1.0	15044.90	0.9	-3.2	15299.78	0.2	-1.5
HS05	BRH1503837	1995.29	0.1	-77.6	9077.58	1.0	1.8	8919.09	2.8	8875.22	0.6	-0.5	9202.84	2.4	3.2
HS06	BRH1503838	1078.62	0.6	-78.4	5019.53	3.2	0.3	5002.28	0.8	5287.42	2.3	5.7	5877.74	3.5	17.5
HS07	BRH1503839	1219.91	2.1	-75.2	4789.79	1.0	-2.5	4911.02	2.2	5106.38	0.6	4.0	5431.78	3.1	10.6
HS08	BRH1503844	593.63	1.9	-78.6	2629.85	0.4	-5.2	2772.98	1.9	2956.28	2.0	6.6	3278.14	5.3	18.2
HS09	BRH1503848	457.02	1.7	-80.6	2260.80	0.4	-4.2	2360.25	0.6	2593.18	0.6	9.9	2895.55	2.2	22.7
HS10	BRH1503850	337.00	0.9	-83.5	1901.73	2.5	-6.7	2038.12	5.5	2149.42	4.9	5.5	2352.03	5.3	15.4
Mean			1.2	-78.9		1.2	-2.5		1.7		1.4	3.0		2.5	10.9

## CONCLUSIONS

A sensitive immunoassay has been successfully validated and deemed suitable for the quantification of GDF8 in human serum samples using ELISA platform.

The validation data demonstrated that the standard calibration model, accuracy, precision, parallelism (dilution linearity and hook effect), reproducibility and detectability, bench-top, refrigerator, freeze/thaw and long-term stability met all validation acceptance criteria. Method BTM-2580-R0 was validated and deemed suitable for the quantification of GDF-8/Myostatin in human serum. The quantitative range of the method was 31.25 - 4000.00 pg/mL with a MRD of 1:8.

Samples reading above the ULOQ could be diluted to a maximum dilution of 1:32. Myostatin levels in ten individual human serum samples were tested in one run at the MRD (1:8). The detected concentration of GDF-8 in these ten lots was between 2000 to 14,000 pg/mL. Myostatin in human serum was stable up to three freeze/thaw cycles, 4 hours when stored at ambient temperature, 50 hours when stored at 4°C (nominal) and up to 120 days when stored at -20°C (nominal) or -70°C (nominal).

## REFERENCES

1. Quantikine ELISA Human GDF-8/Myostatin Immunoassay (DGDF80) Instructions for Use, R&D Systems.

