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colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human FGF12 in serum, plasma and cell culture supernatants.

general information

Catalogue Number	KE00036
Product Name	FGF12 ELISA Kit
Species cross-reactivity	Human FGF12
Range (calibration Range)	0. 5 - 32 ng/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**2257** (Human)
SwissProt	**P61328** (Human)

kit components & storage

Microplate - antibody coated 96-well Microplate (8 wells × 12 strips)	1 plate	Store at -20°C for six months
Standard - 64 ng/bottle; lyophilized*	2 bottles	Store at -20°C for six months
Detection antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 3-e - 30 mL/bottle. For serum and plasma samples	1 bottle	Store at 2-8°C for six months
Sample Diluent PT 3-ef - 30 mL/bottle. For cell culture supernatants	1 bottle	Store at 2-8°C for six months
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Plate Cover Seals	3 pieces	

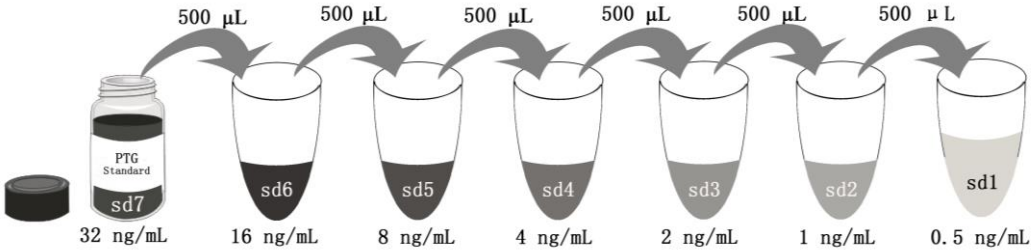
NB: Do not use the kit after the expiration date.

Sample Diluent PT 3-e is for Standard, serum and plasma samples.

Sample Diluent PT 3-ef is for Standard and cell culture supernatants.

Detection Diluent is for Detection antibody and HRP-conjugated antibody.

*Add 2 mL Sample Diluent PT 3-e or PT 3-ef in Standard, This reconstitution gives a stock solution of 32 ng/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-e or PT 3-ef	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00036 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The FGF12 ELISA kit is to be used to detect and quantify protein levels of endogenous FGF12. The assay recognizes human FGF12. A polyclonal antibody specific for FGF12 has been pre-coated onto the microwells. The FGF12 protein in samples is captured by the coated antibody after incubation. Following extensive washing, a monoclonal antibody specific for FGF12 is added to detect the captured FGF12 protein. For signal development, horseradish peroxidase (HRP)-conjugated Anti-mouse antibody 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 450nm.

background

FGF12 is a member of the fibroblast growth factor (FGF) family. FGF family members play important roles in embryogenesis, angiogenesis, and wound repair. FGF12 lacks the N-terminal signal sequence present in most of the FGF family members, but it contains clusters of basic residues that have been demonstrated to act as a nuclear localization signal. When transfected into mammalian cells, this protein accumulated in the nucleus, but was not secreted. FGF12 plays an intracellular role in the inhibition of radiation-induced apoptosis. FGF12 is expressed abundantly in developing and adult nervous systems; therefore, FGF12 was thought to be related to nervous system development and function.

sample preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

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.

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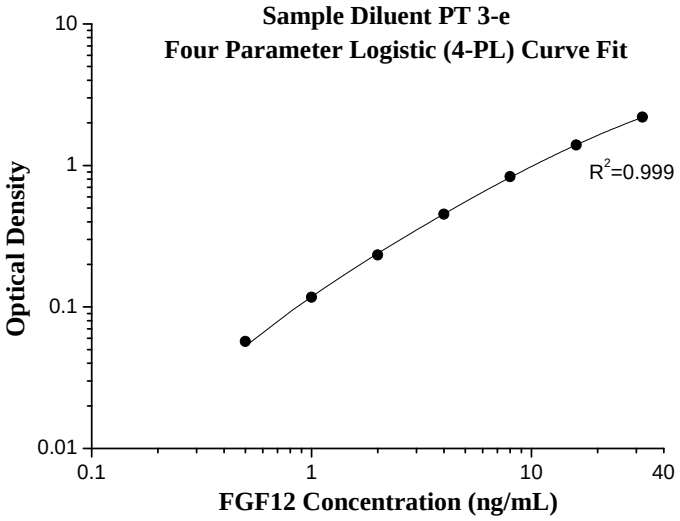
assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	**120 min**	4 times	Cover Wells
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells
4	TMB Substrate	100 µL	15-30 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.				

typical data

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



(ng/mL)	O.D	Average	Corrected
0	0.028 0.029	0.0285	—
0.5	0.083 0.088	0.0855	0.057
1	0.14 0.151	0.1455	0.117
2	0.243 0.28	0.2615	0.233
4	0.456 0.506	0.481	0.4525
8	0.852 0.87	0.861	0.8325
16	1.387 1.456	1.4215	1.393
32	2.186 2.256	2.221	2.1925

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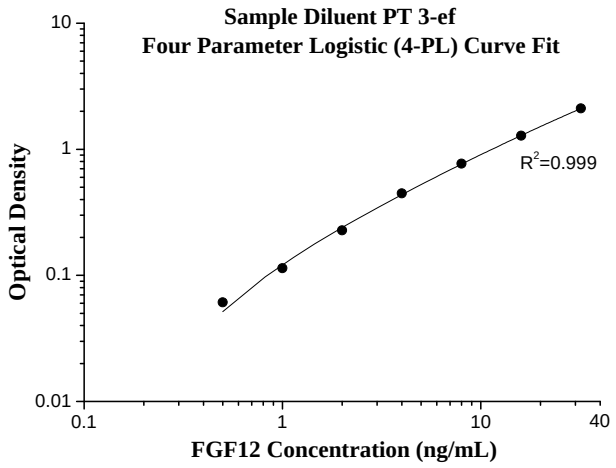

(ng/mL)	O.D	Average	Correcte
0	0.023 0.024	0.0235	—
0.5	0.086 0.083	0.0845	0.061
1	0.139 0.136	0.1375	0.114
2	0.249 0.253	0.251	0.2275
3	0.485 0.455	0.47	0.4465
8	0.779 0.803	0.791	0.7675
16	1.315 1.298	1.3065	1.283
32	2.189 2.073	2.131	2.1075

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			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (ng/ml)	29.36	6.07	1.50	29.30	6.07	1.49
SD	2.1	0.33	0.08	1.8	0.36	0.08
CV%	7.2	5.4	5.1	6.1	6.0	5.3

recovery

The recovery of FGF12 spiked to three different levels in four samples throughout the range of the assay in vrious matrices was evaluated.

Sample Type		Average % of Expected	Range(%)
Citrate plasma	1:2	89	72-100
	1:4	92	76-125
Cell culture supernatants	1:1	103	82-120
	1:2	101	77-120

sensitivity

The minimum detectable dose of human FGF12 is 0.015 ng/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, three samples were spiked with high concentrations of FGF12 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.
(The samples were initially diluted 1:2)

		Citrate plasma (Sample Diluent PT 3-e)	Cell culture supernatants (Sample Diluent PT 3-ef)
1:2	Average% of Expected	85	89
	Range(%)	83-89	88-91
1:4	Average% of Expected	82	99
	Range(%)	79-85	93-104
1:8	Average% of Expected	86	100
	Range(%)	80-91	97-104
1:16	Average% of Expected	90	95
	Range(%)	82-97	88-93

references

1. Smallwood PM. et al. (1996). Proc Natl Acad Sci U S A. 93: 9850-9857.
2. Nakayama F. et al. (2008). J Radiat Res. 49: 491-501.
3. Hartung H. et al.(1997). 64: 31-9.
4. provided by RefSeq, Jul 2008.

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