Human FGF2 Sandwich ELISA Kit Datasheet

For the quantitative detection of Human FGF2 concentrations in cell lysates.

General Information

Catalogue Number	KE00129
Product Name	Human FGF2 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	31.25-1000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	2247
SwissProt	P09038

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 1-ef - 30 mL/bottle	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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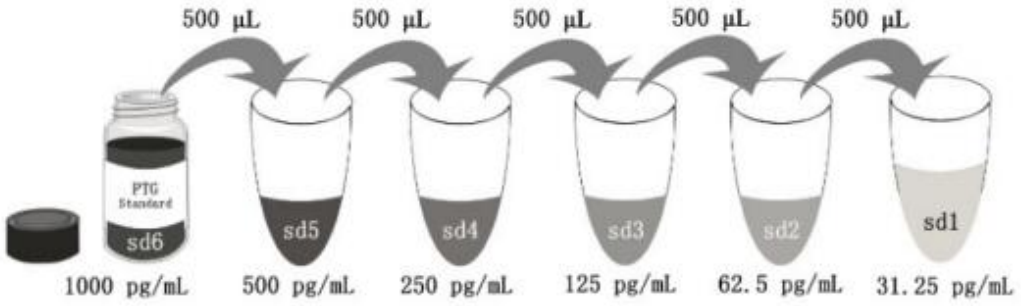
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-ef is for protein standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **2 mL** Sample Diluent PT 1-ef in protein standard. This reconstitution gives a stock solution of 1000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1-ef	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00129 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The FGF2 ELISA kit is to be used to detect and quantify protein levels of endogenous FGF2. The assay recognizes human FGF2. An antibody specific for FGF2 has been pre-coated onto the microwells. The FGF2 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for FGF2 is added to detect the captured FGF2 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

Fibroblast growth factor 2 (FGF2) is one of the most well-studied members of the fibroblast growth factor superfamil. FGF2 can be synthesized and secreted by human adipocytes. FGF2 activates its target receptor tyrosine kinases, the FGFRs, on the cell surface in order to activate numerous downstream pathways, including several mitogen activated protein kinase (MAPK) pathways. FGF2 inhibited TGF β -mediated fibroblast activation, resulting in more rapidly proliferating, spindle-shaped cells, compared to the more slowly proliferating, flatter TGF β -treated cells. FGF2 is an important regulator of cell growth and differentiation under physiological and pathological conditions. FGF2 is widely involved in important biological processes such as stem cell proliferation and angiogenesis.

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Sample Preparation

The cell lysates may require proper dilution to fall within the range of the assay. 1:40 or 1:80 dilution is recommended for Hela cell lysates, 1:4 or 1:8 dilution is recommended for HUVEC and 3T3-L1 cell lysates.

Safety Notes

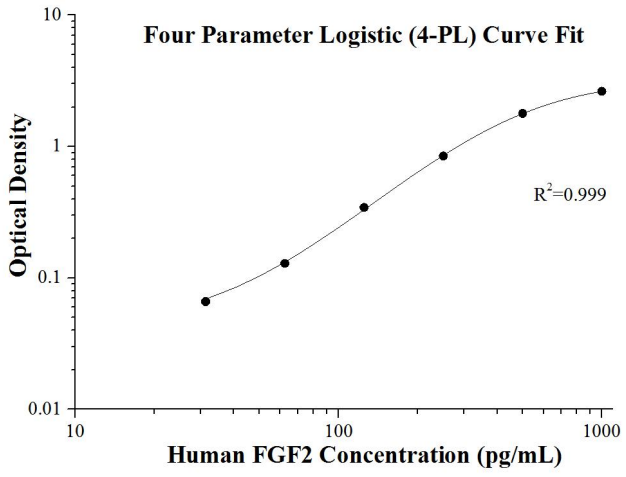
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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.



(pg/mL)	O.D	Average	Corrected
0	0.104 0.109	0.106	-
31.25	0.143 0.202	0.172	0.066
62.5	0.251 0.22	0.235	0.129
125	0.45 0.451	0.450	0.344
250	0.95 0.957	0.953	0.847
500	1.879 1.904	1.891	1.785
1000	2.811 2.668	2.739	2.633

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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	507.6	25.6	5.0
2	20	110.7	6.3	5.7
3	20	62.3	3.0	4.8

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	500.7	21.9	4.4
2	24	114.3	9.8	8.6
3	24	63.3	3.1	4.9

Recovery

The recovery of FGF2 spiked to three different levels in four samples throughout the range of the assay in cell lysates was evaluated.

Sample Type		Average % of Expected	Range (%)
Cell lysates	1:80	100	85-124
	1:160	90	81-96

Sample Values

	FGF2 (pg/mL)	Total protein (mg/mL)
Hela cell lysates	29,649	5
HUVEC cell lysates	7,128	6.5
3T3-L1 cell lysates	864	2.9

Sensitivity

The minimum detectable dose of Human FGF2 is 18.8 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, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The Hela cell lysates was initially diluted 1:20, the HUVEC and 3T3-L1 cell lysates was initially diluted 1:2)

		Cell lysates
1:2	Average% of Expected	84
	Range (%)	75-100
1:4	Average% of Expected	97
	Range (%)	92-100
1:8	Average% of Expected	106
	Range (%)	92-118
1:16	Average% of Expected	87
	Range (%)	75-95

References

1. Lanner F. et al. (2010). Development. 137(20):3351-60.
2. Powers CJ. et al. (2000). Endocr Relat Cancer. 7(3):165-97.
3. Ornitz DM. et al. (2015). Wiley Interdiscip Rev Dev Biol. 4(3):215-66.
Eiselleova L. et al. (2009). Stem Cells. 27(8):1847-57.

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