Human ATF4 Sandwich ELISA Kit Datasheet

For the quantitative detection of Human ATF4 concentrations in cell lysates.

General Information

Catalogue Number	KE00147
Product Name	Human ATF4 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	93.8-6000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	468
SwissProt	P18848

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 - 12000 pg/bottle; lyophilized*	2 bottles	
Detection antibody (100X) - 120 μL/vial	1 vial	
HRP-conjugated antibody (100X) - 120 μL/vial	1 vial	
Sample Diluent PT 4 - 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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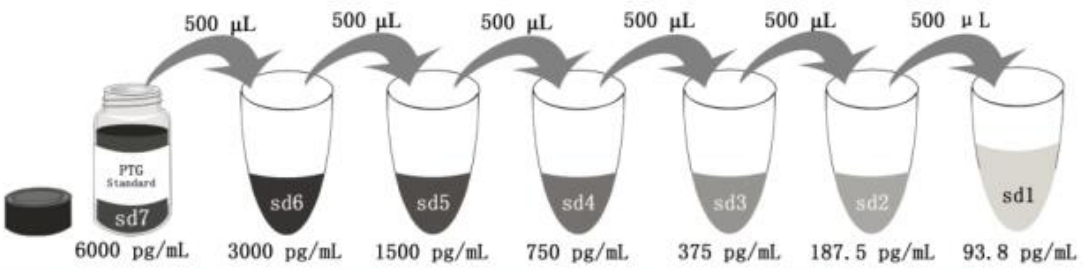
NB: Do not use the kit after the expiration date.
Sample Diluent PT 4 is for protein standard and samples.
Detection Diluent is for Detection antibody and HRP-conjugated antibody.
*Add **2 mL** Sample Diluent PT 4 in protein standard. This reconstitution gives a stock solution of 6000 pg/mL.



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 4	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00147 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The ATF4 ELISA kit is to be used to detect and quantify protein levels of endogenous ATF4. The assay recognizes human ATF4. An antibody specific for ATF4 has been pre-coated onto the microwells. The ATF4 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for ATF4 is added to detect the captured ATF4 protein. For signal development, horseradish peroxidase (HRP)-conjugated 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 450 nm with the correction wavelength set at 630 nm.

Background

ATF4 is a transcription factor, that accumulates predominantly in osteoblasts, where it regulates terminal osteoblast differentiation and bone formation[PMID: 19016586]. As a basic leucine-zipper (bZip) transcription factor, ATF4 can regulate amino acid metabolism, cellular redox state, and anti-stress responses. It also regulates age-related and diet-induced obesity and glucose homeostasis in mammals, and has conserved metabolic functions in flies[PMID: 19726872]. Due to its location at chromosome 22q13, a region linked to schizophrenia, ATF4 is considered as a positional candidate gene for schizophrenia[PMID: 18163433]. Otherwise, since ATF4 is induced by tumour microenvironmental factors, and regulates processes relevant to cancer progression, it might serve as a potential therapeutic target in cancer.

Sample Preparation

The cell lysates samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:4, 1:8 is

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suggested according to the individual samples.

Safety Notes

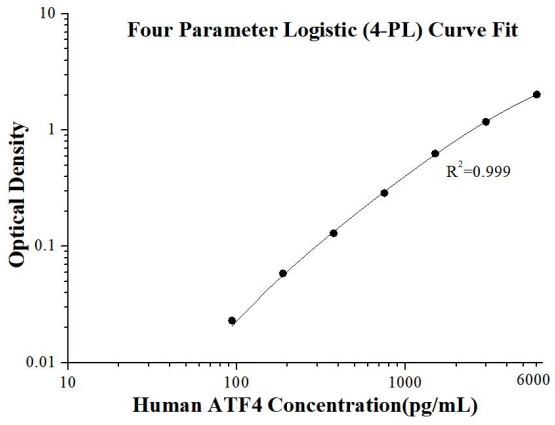
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	0.087 0.087	0.087	-
93.8	0.111 0.109	0.110	0.023
187.5	0.145 0.146	0.146	0.059
375	0.216 0.217	0.217	0.130
750	0.371 0.378	0.375	0.288
1500	0.730 0.699	0.715	0.628
3000	1.268 1.265	1.267	1.180
6000	2.097 2.120	2.109	2.022

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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	279.9	18.0	6.4
2	20	878.4	44.9	5.1
3	20	3,600.8	170.7	4.7

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	290.2	24.1	8.3
2	24	883.5	56.3	6.4
3	24	3,553.9	139.6	3.9

Recovery

The recovery of ATF4 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	1:4	90	76-113
	1:8	103	70-107

Sample Values

HepG2 cells were cultured in DMEM supplemented with 10% fetal bonive serum, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfa
Cells were cultured unstimulated or stimulated with 1 μM Thapsigarin for 5h. Aliquots of the cell culture supernates were removed.
Cells were lysed and measured the total protein, assayed for human ATF4.

HepG2 cell lysates (3.5×10^7 cells/mL)	ATF4 Concentration (pg/mL)	Total protein (mg/mL)
unstimulated	740	2
1 μM TG stimulated for 5h	15,731	2

Sensitivity

The minimum detectable dose of human ATF4 is 77.8 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, cell lysates were diluted with the **Sample Diluent PT 4** to produce samples with values within the dynamic range of the assay. (The cell lysates sample were initially diluted 1:2)

		Cell lysates
1:2	Average% of Expected	100
	Range (%)	-
1:4	Average% of Expected	101
	Range (%)	100-106
1:8	Average% of Expected	98
	Range (%)	97-98
1:16	Average% of Expected	88
	Range (%)	76-91

References

1.Ghosh R, Lipson KL, Sargent KE, Mercurio AM, Hunt JS, et al. (2010) Transcriptional Regulation of VEGF-A by the Unfolded Protein Response Pathway. PLoS ONE 5(3): e9575. doi:10.1371/journal.pone.0009575
2. Su X, Chu Y, Kordower JH, Li B, Cao H, Huang L, et al. (2015) PGC–1α Promoter Methylation in Parkinson's Disease. PLoS ONE 10(8): e0134087. doi:10.1371/journal.pone.0134087

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