

Human ATF4 Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE00147

Size: 96T

Sensitivity: 77.8 pg/mL

Range: 93.8-6000 pg/mL

Usage: For the quantitative detection of human ATF4 concentrations in cell lysate.

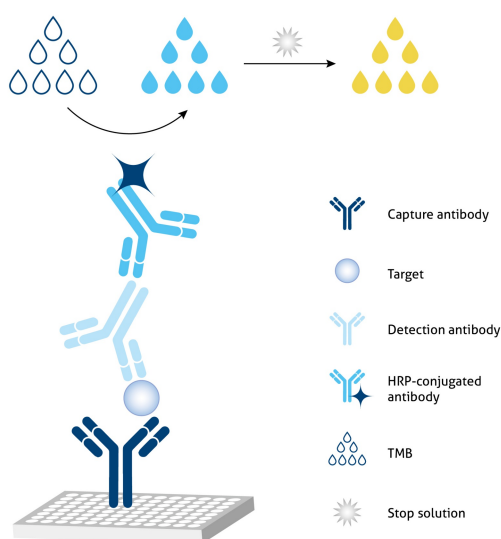
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1. Background

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.

2. Principle



Sandwich ELISA structure (HRP conjugated secondary antibody)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody also binds to the analyte. Horseradish peroxidase (HRP)-conjugated secondary antibody binds to the detection antibody. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

- 3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.
- 3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.
- 3.3 Plate washer: automated or manual.
- 3.4 Absorbent paper towels.
- 3.5 Glass or plastic tubes to prepare standard and sample dilutions.
- 3.6 Beakers and graduated cylinders.
- 3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

4. Kit Components and 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 (100×) - 120 µL/vial*	1 vial	
HRP-conjugated antibody (100×) - 120 µL/vial*	1 vial	
Sample Diluent PT 4 - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 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	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

- 5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.
- 5.2 Do not use the kit after the expiration date.
- 5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.
- 5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.
- 5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

6.1 Cell Lysate:

- 1) Collect cells and wash by centrifuging at 500 × g for 5 minutes before resuspension in pre-cooled PBS buffer. Perform this step three times.
- 2) Count cells and then discard the supernatant.
- 3) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing cell lysis.
- 4) Add 1 mL of Extraction reagent (containing protease inhibitor cocktail) Per 1 × 10⁷ cells, Incubate cell suspension on ice for 30 minutes, use ultrasound to treat the samples.
- 5) Centrifuge cell lysate at 10,000 × g for 5 minutes at 4°C.
- 6) Measure the concentration of total protein in cell lysate using BCA assay. Where possible, keep samples on ice to avoid protein degradation.

7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Detection Antibody (1X): Dilute 100X Detection Antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 μ L 100X Detection Antibody + 990 μ L Detection Diluent (Centrifuge the 100 X Detection Antibody solution for a few seconds prior to use).

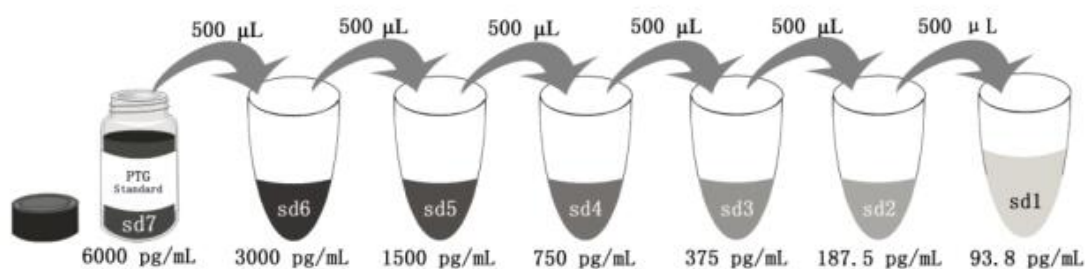
7.3 HRP-conjugated antibody (1X): Dilute 100X HRP-conjugated antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 μ L 100X HRP-conjugated antibody + 990 μ L Detection Diluent (Centrifuge the 100X HRP-conjugated antibody solution for a few seconds prior to use).

7.4 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:4 or 1:8 is recommended for cell lysate.

7.5 Standard Serial Dilution:

Add 2 mL Sample Diluent PT 4 in protein standard.



Add # μ L of Standard diluted in the previous step	—	500 μ L	500 μ L	500 μ L	500 μ L	500 μ L	500 μ L
# μ L of Sample Diluent PT 4	2000 μ L	500 μ L	500 μ L	500 μ L	500 μ L	500 μ L	500 μ L
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody and HRP-conjugated antibody can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group, standard group and sample group, add 100 µL of each standard and sample to the appropriate wells. (Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL of 1X Detection Antibody solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate for 1 hour at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Add 100 µL of 1X HRP-conjugated antibody solution (refer to Reagent Preparation 7.3) to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37°C.

8.8 Repeat wash step in 8.4.

8.9 Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.10 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.11 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

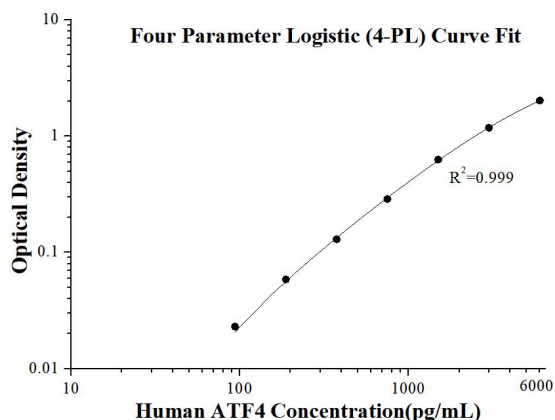
8.12 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve-fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

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.				

9. Validation Data

9.1 Standard curve

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

9.2 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

9.3 Recovery

The recovery of human ATF4 spiked to three different levels throughout the range of the assay in cell lysate was evaluated.

Sample Type		Average% of Expected	Range (%)
Cell lysate	1:4	90	76-113
	1:8	103	70-107

9.4 Sample values

HepG2 cells were cultured in DMEM supplemented with 10% fetal bovine

serum, 100 U/mL penicillin, and 100 µg/mL streptomycin

sulfate. Cells were cultured unstimulated or stimulated with 1µM Thapsigargin for 5h. Aliquots of the cell culture supernates were removed. Cells were lysed and measured the total protein, assayed for human ATF4.

HepG2 cell lysate (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

9.5 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.

9.6 Linearity

To assess the linearity of the assay, cell lysate was diluted with the **Sample Diluent PT 4** to produce samples with values within the dynamic range of the assay. (The cell lysate sample was initially diluted 1:2)

		Cell lysate
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

10. 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