FOR RESEARCH USE ONLY!

05/21

ATF4 (Human) ELISA Kit

(Catalog # E5008-100, 96 assays, Store at 4ºC)

I. Introduction:

Activating transcription factor 4 (ATF4) is 351 amino acid basic leucine zipper (bZIP) stress-responsive transcription factor that belongs to the cAMP response element-binding protein 2 (CREB 2) family of proteins. The protein contains a leucine zipper domain that is responsible for the formation of homo and heterodimers necessary for protein-protein interactions. ATF4 is stimulated under cellular stress conditions such as amino acid depletion by General Control Nonderepressible-2 (GCN2) and protein kinase R-like ER kinase (PERK). Both GCN2 and PERK phosphorylate eIF2α and inhibit global protein translation. However, phosphorylation of eIF2α stimulates ATF4 to switch on nonessential amino acid (NEAA) biosynthesis and promote transportation activity of amino acids to help cells cope with amino acid depletion. ATF4 plays a role in the differentiation of osteoblasts and may provide insights into the progression of skeletal diseases. ATF4 also plays a significant role in diseases such as diabetic retinopathy, Alzheimer's, Parkinson's, Huntington's, and prion disease. BioVision's ATF4 (Human) ELISA kit quantitatively measures ATF4 in human serum, plasma, and other biological fluids. Test samples, Standards, and Biotinylated Detection antibody are added to the wells pre-coated with capture antibody and then washed with Wash Buffer. HRP-Streptavidin is added to the plate, and post-incubation any unattached conjugates are washed off by Wash Buffer. The HRP enzymatic reaction is detected using TMB-substrate. Finally, an acidic stop solution terminates the enzymatic reaction. The color developed is directly proportional to the amount of ATF4 in the sample or standard.

II. Features and Benefits:

- Detection range: 0.16 – 10 ng/ml
- Sensitivity: 0.10 ng/ml
- Assay Precision: Intra-Assay CV and Inter-Assay CV < 10%
- Recovery range: 85 - 110% for normal human serum and plasma samples
- This Sandwich ELISA is highly sensitive and highly specific for the detection of ATF4 in human samples. There is no significant cross-reactivity or interference between ATF4 and analogues

III. Sample Type:

Human Serum, Plasma, Tissue lysates and other biological fluids

IV. Kit Contents:

Components	E5008-100	Part Number	Storage Temp.
Micro ELISA plate	8 x 12 Strips	E5008-100-1	-20°C
Standard (10 ng)	2 vials	E5008-100-2	-20°C
Sample/Standard Dilution Buffer	20 ml	E5008-100-3	4°C
Biotin-labeled Antibody (100X)	120 µl	E5008-100-4	-20°C
Antibody Dilution Buffer	14 ml	E5008-100-5	4°C
HRP Conjugate (100X)	120 µl	E5008-100-6	4°C (Avoid light)
HRP Conjugate Dilution Buffer	14 ml	E5008-100-7	4°C
TMB Substrate Solution	10 ml	E5008-100-8	4°C (Avoid light)
Stop Solution	10 ml	E5008-100-9	4°C
Wash Buffer (25X)	30 ml	E5008-100-10	4°C
Plate Sealers	5	E5008-100-11	4°C

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit can be stored at 4°C for up to 6 months from the date of shipment.

VII. Reagent and Sample Preparation:

Note: Prepare reagents within 30 minutes before the experiment

Before using the kit, spin tubes and bring down all components to the bottom of tubes

1. **Biotin-labeled Antibody working solution:** Prepare this working stock 1 hour prior to the start of the experiment. Calculate the total volume of the working solution: 0.1 ml/well x quantity of wells. Add 0.1 – 0.2 ml to the total volume. Dilute the **Biotin-labeled antibody (100X)** with **Antibody Dilution Buffer** at 1:100. Mix thoroughly.
2. **HRP Conjugate working solution:** Prepare this working stock 30 minutes prior to the start of the experiment. Calculate the total volume of the working solution: 0.1 ml/well x quantity of wells. Add 0.1 – 0.2 ml to the total volume. Dilute the **HRP Conjugate (100X)** with **HRP Conjugate Dilution Buffer** at 1:100. Mix thoroughly.
3. **Wash Buffer (1X):** Dilute **Wash Buffer (25X)** to 1X by adding 30 ml of **Wash Buffer (25X)** and make up the volume to 750 ml with deionized/distilled water. If crystals present in the **Wash Buffer (25X)**, warm it in water bath at 40°C. Mix it gently. The solution must be cooled to room temperature before use.
4. **Standard Preparation:**

- Centrifuge the standard vial at 10,000 x g for 1 minute. Add 1 ml of **Sample/Standard Dilution Buffer**; let it stand for 10 minutes, then mix gently several times. The reconstituted standard has a concentration of **10 ng/ml**.
- Label 7 tubes as **5, 2.5, 1.25, 0.63, 0.32, 0.16 and 0 ng/ml** respectively. Add 0.5 ml of the **Sample/Standard Dilution Buffer** into each tube.
- Transfer 0.5 ml from the reconstituted standard vial (10 ng/ml) to the 1st tube to make a concentration of 5 ng/ml and mix them thoroughly. Transfer 0.5 ml from 2nd tube to 3rd tube and mix them thoroughly, and so on. The 0 ng/ml tube will contain only 0.5 ml of the **Sample/Standard Dilution Buffer**.

5. Sample Preparation:

Note: Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C (≤ 1 month) or -80°C (≤ 2 months). Avoid multiple freeze-thaw cycles. The hemolytic samples are not suitable for this assay.

- **Serum**: Place whole blood sample at room temperature for 2 hours or put it at 2 - 8°C overnight and centrifuge for 15 minutes at approximately 1000xg, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and endotoxin free.
- **Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000xg at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
- **Tissue homogenates**: As hemolytic blood may affect the assay result, it is necessary to remove residual blood by washing tissue with pre-cooling PBS buffer (0.01 M, pH 7.4). Mince tissue after weighing it and homogenize it in PBS (the volume depends on the weight of the tissue. Normally, 9 ml PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000xg to get the supernatant.
- **Cell culture supernatant and other biological fluids**: Centrifuge supernatant for 20 minutes at 1000xg at 2 - 8°C to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately.

Note: End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve should be run for each assay.

1. Prepare all reagents, samples and standards as instructed in **section VII**.
2. Add 100 µl of each **standards** or **samples** into appropriate wells. Cover well and incubate for 1.5 hours at 37ºC.
3. Discard the liquid from each well. **Do not wash the plate**. Add 0.1 ml of **Biotin-Detection antibody work solution** into the above wells. Seal the plate and incubate at 37ºC for 60 minutes.
4. Discard the solution and wash 3 times with **Wash Buffer (1X)**. Wash by filling each well with Wash Buffer (350 µl) using a multi-channel pipette or autowasher. Let it soak for 1-2 minutes, and then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Tap the plate on absorbent filter papers or other absorbent materials.
5. Add 0.1 ml of **HRP Conjugate working solution** into each well, cover the plate and incubate at 37ºC for 30 minutes.
6. Discard the solution and wash 5 times with **Wash Buffer (1X)** as step 4.
7. Add 90 µl of **TMB substrate** into each well, cover the plate and incubate at 37 ºC in dark for 15 minutes. (Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. The reaction can be terminated when apparent gradient appeared in standard wells).
8. Add 50 µl of **Stop Solution** to each well. Read result at 450 nm within 20 minutes.

IX. Calculation:

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

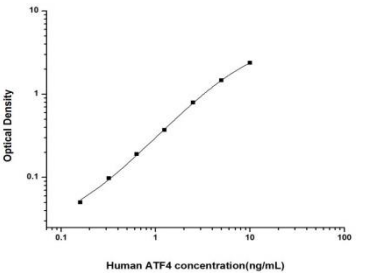

Figure: Typical Standard Curve and OD values: These standard curves are for demonstration only. A standard curve must be run with each assay.

STD. (ng/ml)	OD	Corrected
0	0.091	0.000
0.16	0.141	0.05
0.32	0.189	0.098
0.63	0.281	0.19
1.25	0.462	0.371
2.5	0.882	0.791
5	1.559	1.468
10	2.464	2.373

X. Recovery:

Matrices mentioned below were spiked with certain level of ATF4 and the recovery rates were calculated by comparing the measured value to the expected amount of ATF4 in samples

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	86-101	93
EDTA Plasma (n=5)	86-99	91
Heparin Plasma (n=5)	94-110	101

XI. Linearity:

Linearity of the assay kit was determined by spiking samples and their serial dilutions with appropriate concentration of ATF4. The results are represented as percentage of calculated concentration to the expected value.

Sample	1:2	1:4	1:8	1:16
Serum (n=5)	97-109%	91-103%	91-105%	93-107%
EDTA Plasma (n=5)	94-106%	86-101%	84-99%	80-92%
Cell culture media (n=5)	92-107%	82-94%	88-103%	82-95%

XII. Related Products:

- cAMP ELISA Kit (E4715)
- Osteopontin (OPN) (Human) ELISA Kit (E4920)
- Caspase-9 (Human) ELISA Kit (K4165)
- Osteocalcin (Human) ELISA Kit (E4762)
- MTOR (Human) ELISA Kit (K4179)