SunLong Biotech Co.,LTD
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[Primary Antibody]

Rabbit Anti-ATF4 antibody

Catalog Number: SL1531R

FOR RESEARCH USE ONLY

[DATASHEET]

Host:	Rabbit
Isotype:	IgG
Clonality:	Polyclonal
Target:	ATF4
Immunogen:	KLH conjugated synthetic peptide derived from human ATF4: 251-351/351
Purification:	affinity purified by Protein A
Concentration:	1mg/ml
Storage:	0.01M TBS (pH7.4) with 1% BSA, 0.02% Proclin300 and 50% Glycerol. Shipped at 4℃. Store at -20 °C for one year. Avoid repeated freeze/thaw cycles.
Applications:	WB=1:500-2000 IHC-P=1:100-500 IHC-F=1:100-500 ICC/IF=1:100-500 IF=1:100-500 Flow-Cyt=0.2μg /test （Paraffin sections need antigen repair） not yet tested in other applications. optimal dilutions/concentrations should be determined by the end user.
Reactivity:	Human, Mouse, Rat, (predicted: Dog, Pig, Cow, Horse, Rabbit, Sheep)
Predicted MW.:	38kDa
Subcellular Location:	The nucleus cytoplasmic The cell membrane
Background:	This gene encodes a transcription factor that was originally identified as a widely expressed mammalian DNA binding protein that could bind a tax-responsive enhancer element in the LTR of HTLV-1. The encoded protein was also isolated and characterized as the cAMP-response element binding protein 2 (CREB-2). The protein encoded by this gene belongs to a family of DNA-binding proteins that includes the AP-1 family of transcription factors, cAMP-response element binding proteins (CREBs) and CREB-like proteins. These transcription factors share a leucine zipper region that is involved in protein-protein interactions, located C-terminal to a stretch of basic amino acids that functions as a DNA binding domain. Two alternative transcripts

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	encoding the same protein have been described. Two pseudogenes are located on the X chromosome at q28 in a region containing a large inverted duplication. [provided by RefSeq, Sep 2011] **Function:** Transcriptional activator. Binds the cAMP response element (CRE) (consensus: 5'-GTGACGT[AC][AG]-3'), a sequence present in many viral and cellular promoters. Cooperates with FOXO1 in osteoblasts to regulate glucose homeostasis through suppression of beta-cell production and decrease in insulin production. It binds to a Tax-responsive enhancer element in the long terminal repeat of HTLV-I. Regulates the induction of DDIT3/CHOP and asparagine synthetase (ASNS) in response to ER stress. In concert with DDIT3/CHOP, activates the transcription of TRIB3 and promotes ER stress-induced neuronal apoptosis by regulating the transcriptional induction of BBC3/PUMA. Activates transcription of SIRT4. **Subunit:** Binds DNA as a homo- or heterodimer. Interacts (via its leucine zipper domain) with GABBR1 and GABBR2 (via their C-termini). Interacts (via its DNA binding domain) with FOXO1 (C-terminal half); the interaction occurs in osteoblasts and regulates glucose homeostasis through suppression of beta-cell proliferation and a decrease in insulin production. Interacts with SATB2; the interaction results in enhanced DNA binding and transactivation by these transcription factors. Interacts with CEP290 (via an N-terminal region). Interacts with NEK6, DAPK2 (isoform 2) and ZIPK/DAPK3. Forms a heterodimer with TXLNG in osteoblasts. Interacts with DDIT3/CHOP. **Subcellular Location:** Cytoplasm. Cell membrane. Nucleus. Cytoplasm, cytoskeleton, microtubule organizing center, centrosome. Note=Colocalizes with GABBR1 in hippocampal neuron dendritic membranes. Co- localizes with NEK6 in the centrosome. **Post-translational modifications:** Ubiquitinated by SCF(BTRC) in response to mTORC1 signal, followed by proteasomal degradation and leading to down-regulate expression of SIRT4. Phosphorylated by NEK6. Phosphorylated on the betaTrCP degron motif at Ser-219, followed by phosphorylation at Thr-213, Ser-224, Ser-231, Ser-235 and Ser-248, promoting interaction with BTRC and ubiquitination. Phosphorylation is promoted by mTORC1. Phosphorylated by NEK6. **Similarity:** Belongs to the bZIP family. Contains 1 bZIP (basic-leucine zipper) domain. **Database links:** **Entrez Gene:** 468 Human **Entrez Gene:** 11911 Mouse **Entrez Gene:** 79255 Rat

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	NCBI: 33469976 Human Omim: 604064 Human **SwissProt:** P18848 Human **SwissProt:** Q96AQ3 Human **SwissProt:** Q06507 Mouse **SwissProt:** Q5U4B2 Mouse **SwissProt:** Q8CF69 Mouse **SwissProt:** Q9ES19 Rat **Unigene:** 496487 Human **Unigene:** 641 Mouse **Unigene:** 2423 Rat

[VALIDATION IMAGES]

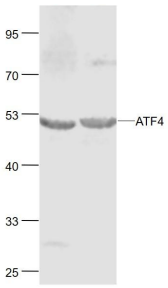


Figure 1. Sample: Cerebrum (Rat) Lysate at 40 ug Cerebrum (Mouse) Lysate at 40 ug Primary: Anti-ATF4 (SL1531R) at 1/1000 dilution Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution Predicted band size: 38 kD Observed band size: 47 kD

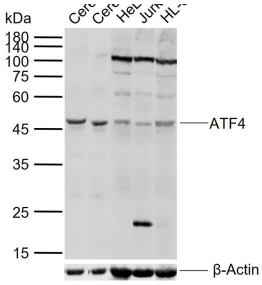


Figure 2. Sample: Lane 1: Mouse Cerebrum tissue lysates Lane 2: Rat Cerebrum tissue lysates Lane 3: Human HeLa cell lysates Lane 4: Human Jurkat cell lysates Lane 5: Human HL-60 cell lysates Primary: Anti-ATF4 (SL1531R) at 1/1000 dilution Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution Predicted band size: 38 kDa Observed band size: 47 kDa

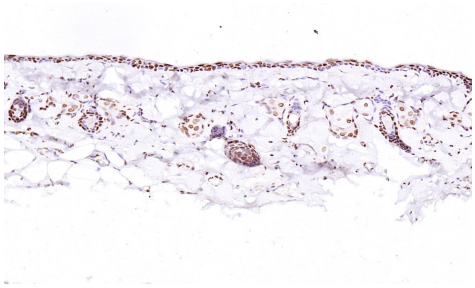


Figure 3. Paraformaldehyde-fixed, paraffin embedded (rat skin); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (SL1531R) at 1:100 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining

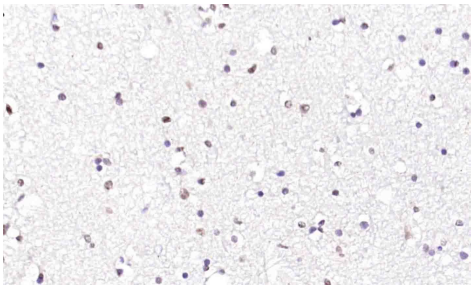


Figure 4. Paraformaldehyde-fixed, paraffin embedded (Human brain); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (SL1531R) at 1:200 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining

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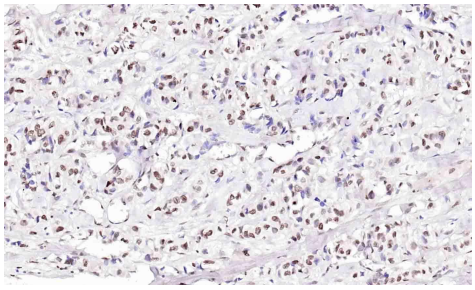


Figure 5. Paraformaldehyde-fixed, paraffin embedded (human breast carcinoma); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (SL1531R) at 1:200 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining

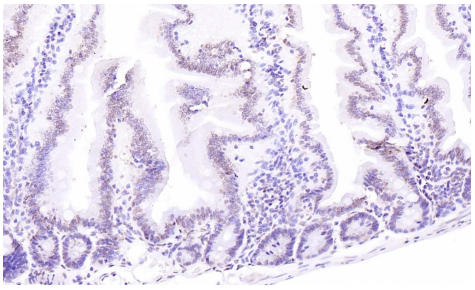


Figure 6. Paraformaldehyde-fixed, paraffin embedded (mouse intestine); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (SL1531R) at 1:100 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining

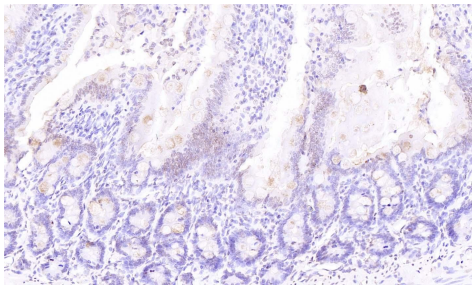


Figure 7. Paraformaldehyde-fixed, paraffin embedded (rat intestine); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (SL1531R) at 1:100 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining

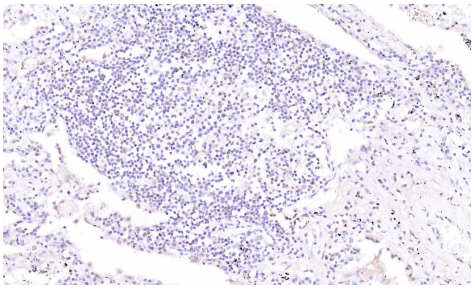


Figure 8. Paraformaldehyde-fixed, paraffin embedded (human lung carcinoma); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (SL1531R) at 1:100 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining

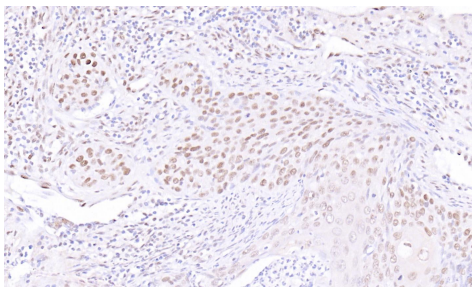


Figure 9. Paraformaldehyde-fixed, paraffin embedded (human cervical carcinoma); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (SL1531R) at 1:100 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining

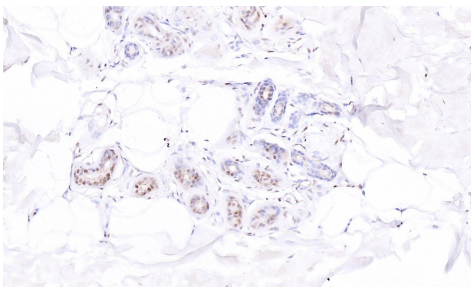


Figure 10. Paraformaldehyde-fixed, paraffin embedded (human Abdominal skin); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (SL1531R) at 1:100 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining

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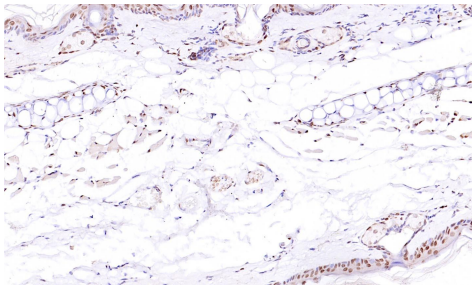


Figure 11. Paraformaldehyde-fixed, paraffin embedded (mouse skin); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (SL1531R) at 1:100 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining

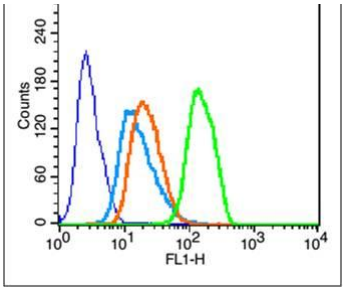


Figure 12. Blank control (blue line): Hela Primary Antibody (green line): Rabbit Anti-Bid antibody (SL1531R) Dilution: 0.2µg /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody (white blue line): Goat anti-rabbit IgG-FITC Dilution: 1µg /test. Protocol The cells were fixed with 70% ethanol (Overnight at 4°C) and then permeabilized with 90% ice-cold methanol for 30 min on ice. Cells stained with Primary Antibody for 30 min at room temperature. The cells were then incubated in 1 X PBS/2%BSA/10% goat serum to block non-specific protein-protein interactions followed by the antibody for 15 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed

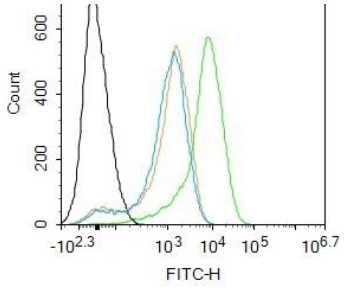


Figure 13. Blank control:Mouse spleen. Primary Antibody (green line): Rabbit Anti-ATF4 Alpha antibody (SL1531R) Dilution: 2µg /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody: Goat anti-rabbit IgG-FITC Dilution: 1µg /test. Protocol The cells were fixed with 4% PFA (10min at room temperature)and then permeabilized with 90% ice-cold methanol for 20 min at -20°C. The cells were then incubated in 5%BSA to block non-specific protein-protein interactions for 30 min at room temperature.Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed

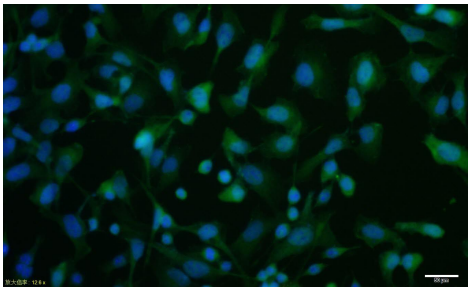


Figure 14. U2OS cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum, C-0005) at 37°C for 20 min; Antibody incubation with (ATF4) polyclonal Antibody, Unconjugated (SL1531R) 1:100, 90 minutes at 37°C; followed by a conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue, C02-04002) was used to stain the cell nuclei

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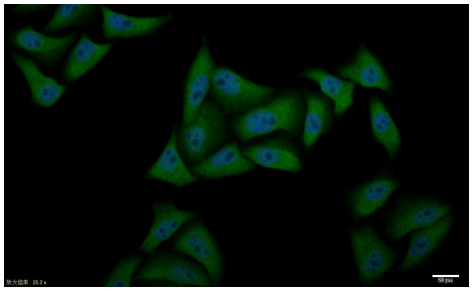


Figure 15. Hela cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum, C-0005) at 37°C for 20 min; Antibody incubation with (ATF4) polyclonal Antibody, Unconjugated (SL1531R) 1:100, 90 minutes at 37°C; followed by a conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue, C02-04002) was used to stain the cell nuclei

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