Data Sheet (Cat.No.TMAB-00159)

Anti-ATF-4 Polyclonal Antibody

Product Details

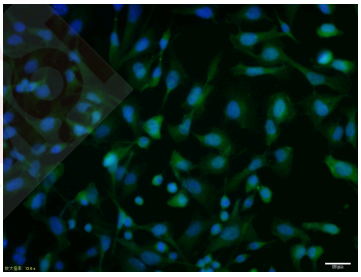
Ig Type:	IgG
Reactivity:	Human,Mouse,Rat (predicted:Dog,Pig,Cow,Horse,Rabbit,Sheep)
Molecular Weight:	Theoretical: 38 kDa. Actual: 47 kDa.
Purification:	Protein A purified

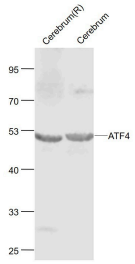
Applications

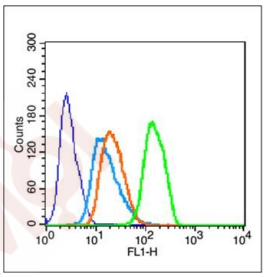
Verified Activity:	1. U2OS cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum) at 37°C for 20 min; Antibody incubation with (ATF4) polyclonal Antibody, Unconjugated (TMAB-00159) 1:100, 90 minutes at 37°C; followed by a conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue) was used to stain the cell nucleus. 2. Sample: Cerebrum (Rat) Lysate at 40 µg Cerebrum (Mouse) Lysate at 40 µg Primary: Anti-ATF4 (TMAB-00159) at 1/1000 dilution Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution Predicted band size: 37/50 kDa Observed band size: 50 kDa 3. Blank control (blue line): Hela Primary Antibody (green line): Rabbit Anti-Bid antibody (TMAB-00159) Dilution: 0.2 µg/10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody (white blue line): Goat anti-rabbit IgG-FITC Dilution: 1 µg/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. 4. Blank control: Mouse spleen. Primary Antibody (green line): Rabbit Anti-ATF4 Alpha antibody (TMAB-00159) Dilution: 2 µg/10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody: Goat anti-rabbit IgG-FITC Dilution: 1 µg/test. Protocol The cells were fixed with 4% PFA (10 min 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. 5. Hela cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum) at 37°C for 20 min; Antibody incubation with (ATF4)

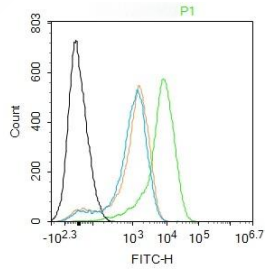
polyclonal Antibody, Unconjugated (TMAB-00159) 1:100, 90 minutes at 37°C; followed by a conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue) was used to stain the cell nucleus.
6. Paraformaldehyde-fixed, paraffin embedded (Human brain); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (TMAB-00159) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructionsand DAB staining.
7. Paraformaldehyde-fixed, paraffin embedded (human breast carcinoma); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (TMAB-00159) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructionsand DAB staining.
8. Paraformaldehyde-fixed, paraffin embedded (mouse skin); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (TMAB-00159) at 1:100 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructionsand DAB staining.
9. Paraformaldehyde-fixed, paraffin embedded (mouse intestine); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (TMAB-00159) at 1:100 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructionsand DAB staining.
10. Paraformaldehyde-fixed, paraffin embedded (rat skin); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (TMAB-00159) at 1:100 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructionsand DAB staining.
11. Paraformaldehyde-fixed, paraffin embedded (rat intestine); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (TMAB-00159) at 1:100 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructionsand DAB staining.
12. Paraformaldehyde-fixed, paraffin embedded (human lung carcinoma); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (TMAB-00159) at 1:100 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructionsand DAB staining.
13. Paraformaldehyde-fixed, paraffin embedded (human cervical carcinoma); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (TMAB-00159) at 1:100 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructionsand DAB staining.
14. Paraformaldehyde-fixed, paraffin embedded (human Abdominal skin); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (TMAB-00159) at 1:100 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructionsand DAB staining.
15. Sample:

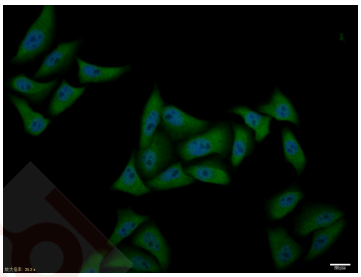
Lane 1: Mouse Cerebrum tissue lysates
Lane 2: Rat Cerebrum tissue lysates
Lane 3: Human HeLa cell lysates
Lane 4: Human Jurkat cell lysates
Lane 5: Human HL-60 cell lysates
Primary: Anti-ATF4 (TMAB-00159) at 1/1000 dilution
Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution
Predicted band size: 38 kDa
Observed band size: 47 kDa

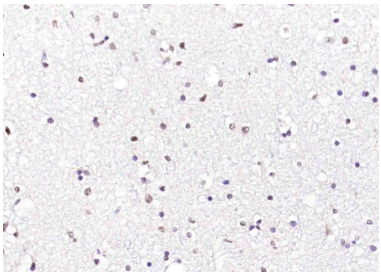


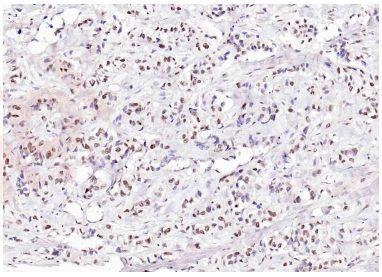


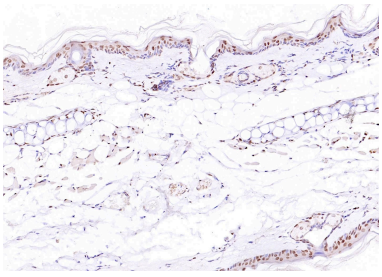


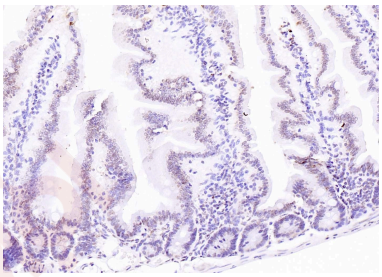


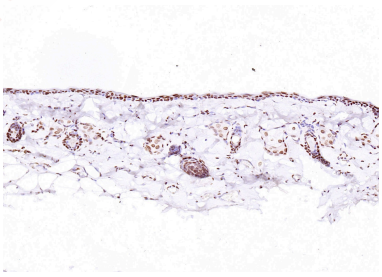


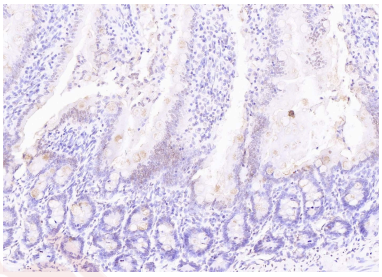


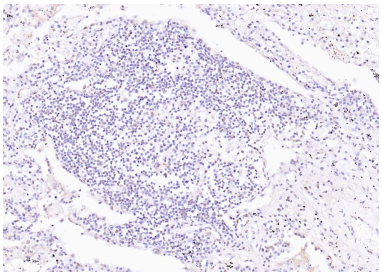


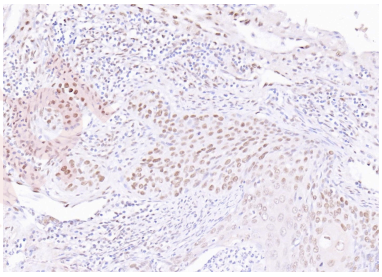


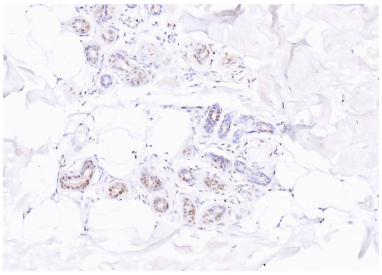


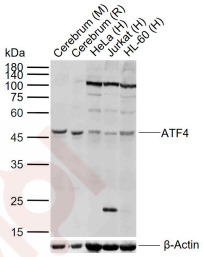












Application:	FCM,ICC/IF,IF,IHC-Fr,IHC-P,WB
Recommended	FCM=0.2 μg/Test; ICC/IF=1:100-500; IF=1:100-500; IHC-Fr=1:100-500; IHC-P=1:100-500; WB=1:500-2000

Properties

Stability & Storage:	Store at -20°C or -80°C for 12 months. Avoid repeated freeze-thaw cycles.
Shipping:	Shipping with blue ice.

Antigen Details

Immunogen:	KLH conjugated synthetic peptide: human ATF4
Antigen Species:	Human
Gene ID:	468
Uniprot ID:	P18848
Synonyms:	Tax Responsive Enhancer Element B67;DNA binding protein TAXREB67;TXREB;Activating Transcription Factor 4;ATF 4;Cyclic AMP response element binding protein 2;Cyclic AMP dependent transcription factor ATF 4;ATF4;CREB2;TAXREB67;CREB 2

Research Background

ATF4 is 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 HTLV1. The encoded protein was also isolated and characterized as the cAMP-response element binding protein 2 (CREB2). The protein encoded by this gene belongs to a family of DNA-binding proteins that includes the AP1 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 (referenced from Entrez gene).