

## Product Datasheet

### ATF4 Rabbit Polyclonal Antibody (orb10155)

<b>Catalog Number</b>	orb10155
<b>Category</b>	Antibodies
<b>Description</b>	ATF4 Rabbit Polyclonal Antibody
<b>Target</b>	ATF4
<b>Clonality</b>	Polyclonal
<b>Species/Host</b>	Rabbit
<b>Isotype</b>	IgG
<b>Conjugation</b>	Unconjugated
<b>Reactivity</b>	Human, Mouse, Rat
<b>Predicted Reactivity</b>	Bovine, Canine, Equine, Porcine, Rabbit, Sheep
<b>Form/Appearance</b>	Liquid
<b>Concentration</b>	1mg/ml
<b>Buffer/Preservatives</b>	0.01M TBS (pH7.4) with 1% rAlbumin, 0.02% Proclin300 and 50% Glycerol.
<b>Purification</b>	Affinity purified by Protein A
<b>Immunogen</b>	KLH conjugated synthetic peptide derived from human ATF4 (251-351/351aa)
<b>UniProt ID</b>	<b>P18848</b>
<b>RRID</b>	AB_10747822
<b>MW</b>	38 kDa

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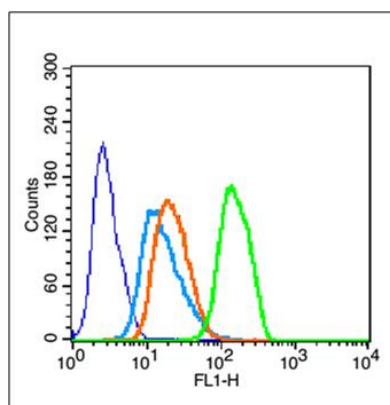
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<b>Tested applications</b>	FC, ICC, IF, IHC-Fr, IHC-P, WB
<b>Dilution range</b>	WB=1:500-2000, IHC-P=1:100-500, IHC-F=1:100-500, ICC/IF=1:100-500, IF=1:100-500, Flow-Cyt=0.2µg /test
<b>Antibody Type</b>	Primary Antibody
<b>Storage</b>	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
<b>Note</b>	For research use only
<b>Expiration Date</b>	12 months from date of receipt.



Blank control (blue line): HeLa, Primary Antibody (green line): Rabbit Anti-Bid antibody (orb10155), Dilution: 0.2 µg/10<sup>6</sup> 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. Acquisition of 20000 events was performed.

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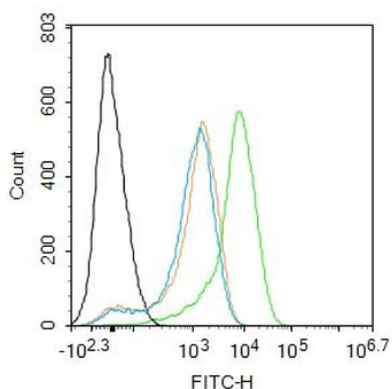
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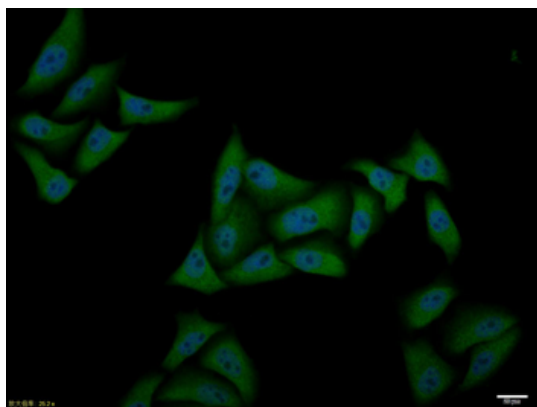
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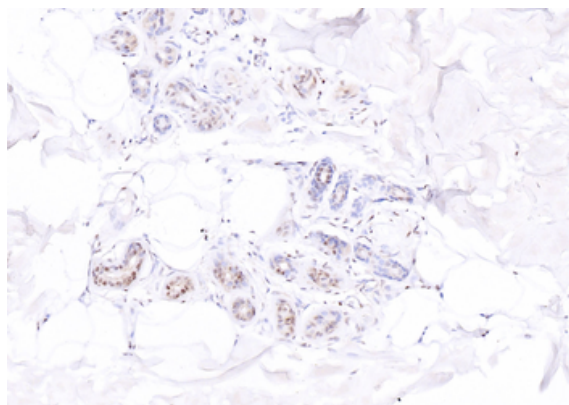
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Blank control: Mouse spleen. Primary Antibody (green line): Rabbit Anti-ATF4 Alpha antibody (orb10155), Dilution: 2  $\mu\text{g}/10^6$  cells, Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody: Goat anti-rabbit IgG-FITC, Dilution: 1  $\mu\text{g}/\text{test}$ . 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  $-20^\circ\text{C}$ . 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 20000 events was performed.



Hela cell, 4% Paraformaldehyde-fixed, Triton X-100 at room temperature for 20 min, Blocking buffer (normal goat serum) at  $37^\circ\text{C}$  for 20 min, Antibody incubation with (ATF4) polyclonal Antibody, Unconjugated (orb10155) 1:100, 90 minutes at  $37^\circ\text{C}$ , followed by a conjugated Goat Anti-Rabbit IgG antibody at  $37^\circ\text{C}$  for 90 minutes, DAPI (blue) was used to stain the cell nuclei.



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 minutes, Blocking buffer (normal goat serum) at  $37^\circ\text{C}$  for 30 min, Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (orb10155) at 1:100 overnight at  $4^\circ\text{C}$ , followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

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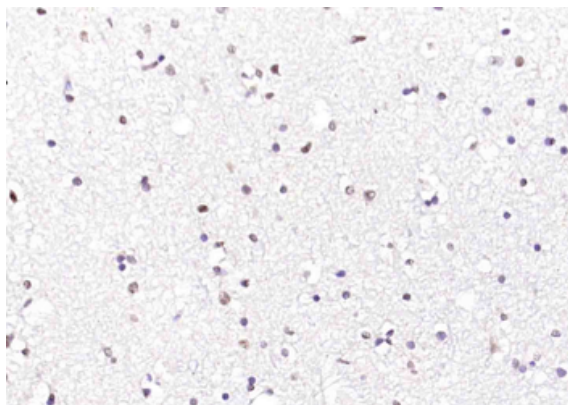
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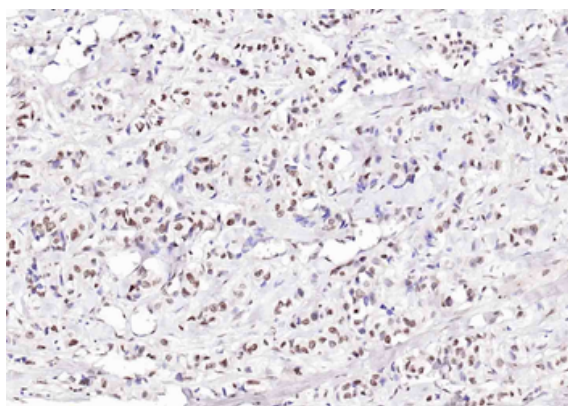
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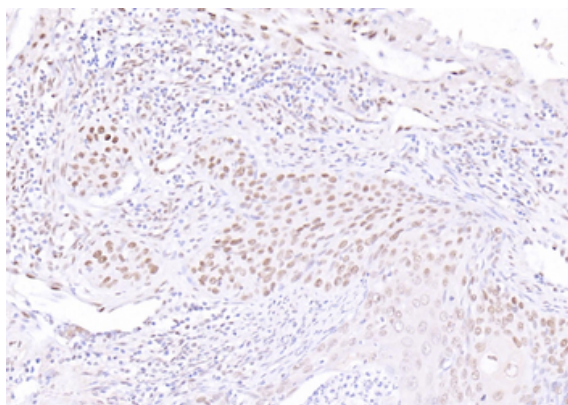
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Paraformaldehyde-fixed, paraffin embedded (Human brain), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (orb10155) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



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 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (orb10155) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



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 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (orb10155) at 1:100 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

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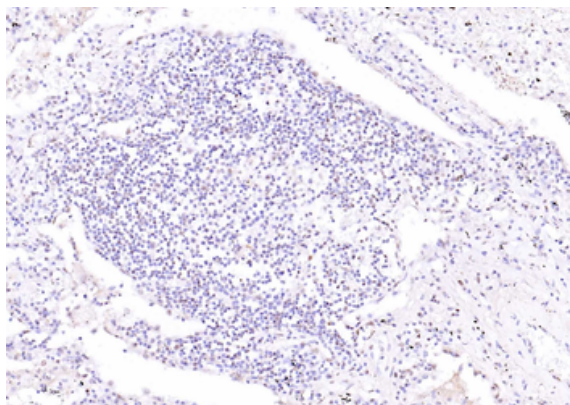
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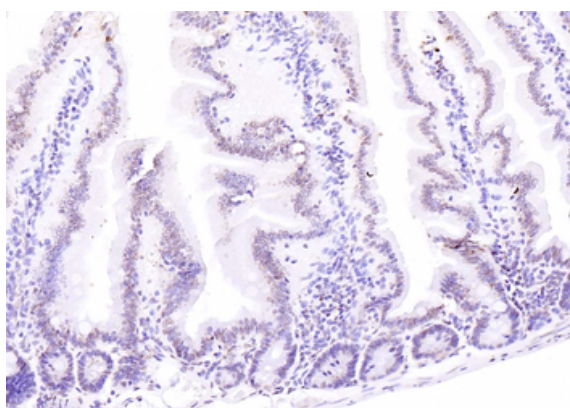
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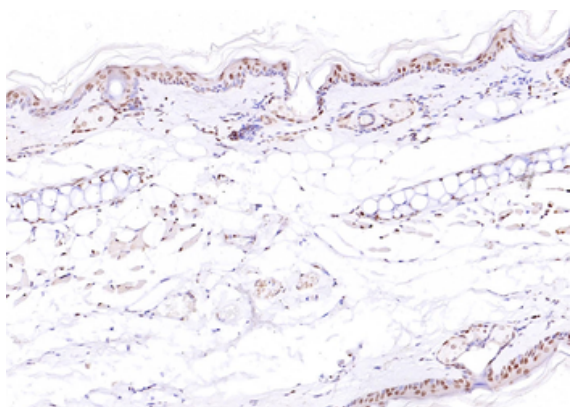
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Paraformaldehyde-fixed, paraffin embedded (human lung carcinoma), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (orb10155) at 1:100 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



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 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (orb10155) at 1:100 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



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 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (orb10155) at 1:100 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

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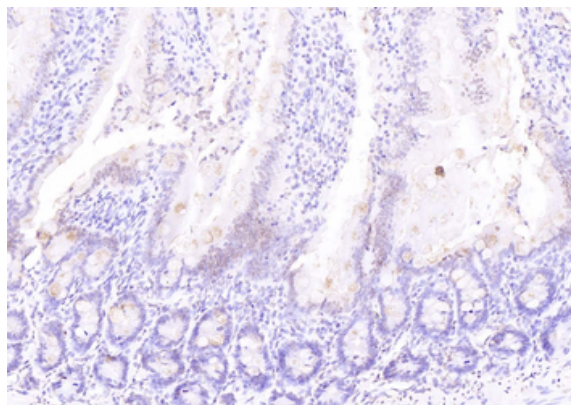
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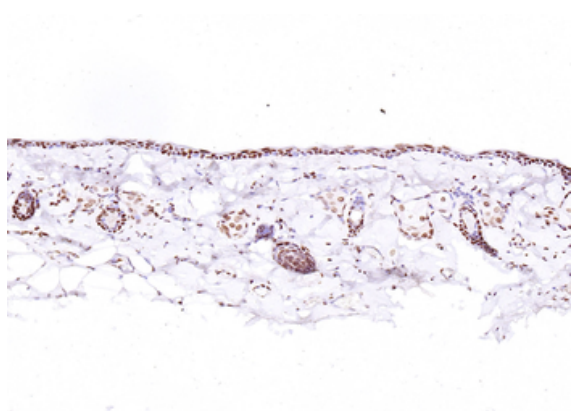
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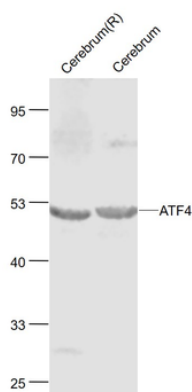
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Paraformaldehyde-fixed, paraffin embedded (rat intestine), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (orb10155) at 1:100 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



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 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (ATF4) Polyclonal Antibody, Unconjugated (orb10155) at 1:100 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



Sample: Cerebrum (Rat) Lysate at 40 ug, Cerebrum (Mouse) Lysate at 40 ug, Primary: Anti-ATF4 (orb10155) at 1/1000 dilution, Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution, Predicted band size: 37'50 kD, Observed band size: 50 kD.

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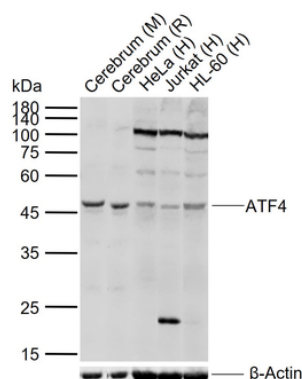
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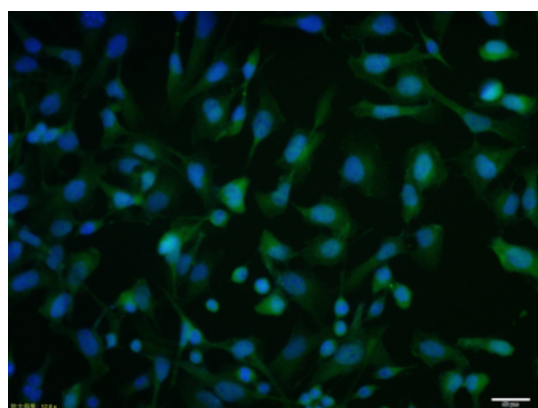
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Sample: Lane 1: Mouse Cerebrum tissue lysates, Lane 2: Rat Cerebrum tissue lysates, Lane 3: Human HeLa cell lysates, Lane 4: Human Jurkat cell lysates, Lane 5: Human HL-60 cell lysates, Primary: Anti-ATF4 (orb10155) at 1/1000 dilution, Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution, Predicted band size: 38 kDa, Observed band size: 47 kDa.



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 (orb10155) 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 nuclei.

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