

ATF4 Ready-To-Use IHC Kit

Cat.No: IHC0128
Applications: **IHC-P**
Reactivity: Human, Mouse, Rat
Size: 50T
Assay type: Immunohistochemistry
Sample type: FFPE tissue
General Information:

Number	Component	Size	Concentration	Storage
1	PBS Buffer (powder)	2 L×2	20x	RT
2	Antigen Retrieval Buffer	20 ml	100x	2-8°C
3	Endogenous Peroxidase Blocking Buffer	3 ml	RTU	2-8°C, protect from light
4	Blocking Buffer	3 ml	RTU	2-8°C
5	Primary Antibody (ATF4 Rabbit pAb)	6 ml	RTU	2-8°C
6	Secondary Antibody (HRP-Goat anti-Rabbit IgG pAb)	6 ml	RTU	2-8°C
7	Chromogen Component A	0.3 ml	RTU	-20°C, protect from light
8	Chromogen Component B	0.3 ml	RTU	-20°C
9	Counter Staining Reagent	5 ml	RTU	RT
10	Mounting Media	5 ml	RTU	RT
11	Control slide (人宫颈癌, 大鼠皮肤, 小鼠皮肤)	3 slides	RTU	RT
12	Datasheet	1 copy		

Immunohistochemistry Protocol:

1. Deparaffinization And Rehydration

Immerse slides in fresh xylene for 15 minutes and then repeat two more times using separate containers. Immerse slides sequentially in 100%, 95%, 90%, 80%, and 70% ethanol solutions for 5 minutes each. Rinse slides 3 times with distilled water for 5 minutes each.

2. Antigen Retrieval

Add 100× **Antigen Retrieval Buffer** into distilled water to prepare a 1× solution. Boil slides in 1× solution at 95°C-100°C for 15 minutes. Move the slides to 1× solution at room temperature (RT) and allow them to stand for 20 minutes. Rinse 3 times with **PBS Buffer** (dissolve the powder in 2L distilled water) for 5 minutes each.

3. Block Endogenous Peroxidase

Drain the liquid off the slides and then use a hydrophobic IHC pen to draw circles on the slides around tissue sections. Add 2-4 drops of **Endogenous Peroxidase Blocking Buffer** directly on slides, covering the whole tissue and block slides for 15 minutes at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

4. Serum Blocking

Block with 2-4 drops of **Blocking Buffer** for 20 minutes at RT.

5. Primary Antibody Incubation

Drain blocking buffer from slides. Incubate slides with 2-4 drops of **ATF4 Rabbit pAb** overnight at 4°C or 1-2 hours at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

6. Secondary Antibody Incubation

Incubate slides with 2-4 drops of **HRP-Goat anti-Rabbit IgG pAb** for 1-2 hours at RT. Rinse slides 3 times with **PBS Buffer** for 5 minutes each.

7. Signal Development

Remove residual liquid around the tissue section. Add 50ul fresh **DAB Buffer (Chromogen Component A : Chromogen Component B : PBS Buffer=1:1:18)** to cover the tissue. Monitor the reaction under the microscope until a brown color is visible (approximate 3-5 minutes at RT). Stop reaction immediately by rinsing with distilled water. Rinse slides 3 times with distilled water for 5 minutes each.

8. Counterstain

Counterstain with an appropriate amount of **Counter Staining Reagent** for 3-5 minutes at RT. Rinse slides with distilled water for 5 minutes. Use 2-4 drops of **Differentiation reagent** to cover the tissue for 30 seconds. Rinse slides twice with distilled water for 5 minutes each.

9. Dehydration Sheet

Immerse slides sequentially in 70%, 80%, 90%, 95%, and 100% ethanol for 5 minutes each at RT. Immerse slides in 2 changes of fresh xylene, 15 minutes each. Drop some **Mounting Media** on the tissue. Mount coverslips.

Notes:

1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.
3. Please don't replace the matching reagents in this product with other

manufacturers' products.

4. As DAB is a carcinogen, please take necessary precautions.

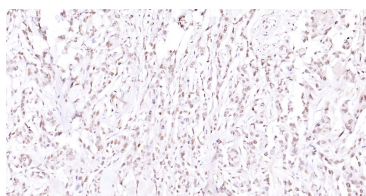
5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1× reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as "IHC0128, Bioss Antibodies". Citation example: "Tissue sections using ATF4 IHC Kit (IHC0128, Bioss Antibodies) were stained for ATF4 according to the manufacturer's instructions."

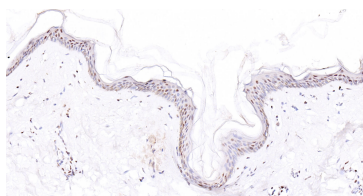
Introduction:

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 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.

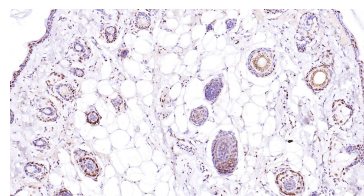
Validation Data



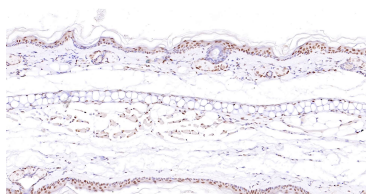
Immunohistochemical analysis of paraffin embedded human skin carcinoma tissue slide using IHC0128 (ATF4 IHC Kit).



Immunohistochemical analysis of paraffin embedded human abdominal skin tissue slide using IHC0128 (ATF4 IHC Kit).



Immunohistochemical analysis of paraffin embedded rat skin tissue slide using IHC0128 (ATF4 IHC Kit).



Immunohistochemical analysis of paraffin embedded mouse skin tissue slide using IHC0128 (ATF4 IHC Kit).