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# Mouse ATF4 Ready-To-Use IHC Kit

Cat.No: IHC0128M
Applications: IHC-P
Reactivity: Mouse
Size: 50T

Assay type: Immunohistochemistry

Sample type: FFPE tissue

General Information:

Number	Component	Size	Concentration	Storage
1	PBS Buffer (powder)	2L×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 (Mouse 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 (小鼠皮肤)	1 slide	RTU	RT
12	Datasheet	1 сору		

# Immunohistoche mistry 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  $100 \times$  **Antigen Retrieval Buffer** into distilled water to prepare a  $1 \times$  solution. Boil slides in  $1 \times$  solution at 95°C-100°C for 15 minutes. Move the slides to  $1 \times$  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 **Mouse 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  $4^{\circ}$ C after preparation; The antigen retrieval buffer ( $1 \times$  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 "IHC0128M, Bioss Antibodies". Citation example: "Mouse

Tissue sections using ATF4 IHC Kit (IHC0128M, 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 chromsome at q28 in a region containing a large inverted duplication.

## Validation Data



Immunohistochemical analysis of paraffin embedded mouse brain tissue slide using IHC0128M (Mouse ATF4 IHC Kit).