

## Mouse Phospho-CREB-1 (Ser133) Ready-To-Use IHC Kit

Cat.No: IHC0345M  
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)	2 L X 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 Phospho-CREB-1 (Ser133) Recombinant Rabbit mAb)	6 ml	RTU	2-8°C
6	Secondary Antibody (Goat Anti-Rabbit IgG H&L, HRP conjugated)	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 (Mouse testis)	1 slide	RTU	RT
12	Datasheet	1 copy		

**Storage and Stability:** Please store components at the temperatures indicated on the individual tube labels. The kit is stable for 6 months from the date of receipt.

**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 **Mouse Phospho-CREB-1 (Ser133) Recombinant Rabbit mAb** 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 **Goat Anti-Rabbit IgG H&L, HRP conjugated** 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.

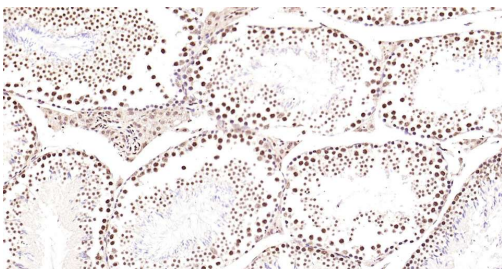
***Please cite this product as "IHC0345M, Bioss Antibodies". Citation example: "Mouse Tissue sections using CREB1 IHC Kit (IHC0345M, Bioss Antibodies) were stained for CREB1 according to the manufacturer's instructions."***

## Introduction:

CREB (Cyclic AMP response element binding protein) is a 43 kDa basic/leucine zipper transcription factor that binds the cyclic AMP response element (CRE) and activates transcription in response to a variety of extracellular signals including neurotransmitters, hormones, membrane depolarization, and growth and neurotrophic factors. Activation of CREB is dependent upon the phosphorylation of serine 133. Phosphorylation of CREB occurs via p44/42 MAP kinase and p90RSK, and via p38 MAP kinase and MSK1. Although CREB will bind DNA independent of its phosphorylation state, only the phosphorylated form is competent as a transcription factor. CREB binding protein (CBP), a transcriptional coactivator that directly interacts with CREB, binds to CREB in the region of serine 133. CREB is involved in different cellular processes including the synchronization of circadian rhythmicity, differentiation of adipose cells, and learning and memory. In humans, the gene is located on the q arm of chromosome 2. Diseases associated with CREB dysfunction include Alzheimer's Disease, histiocytoma and soft tissue melanoma.

## Validation Data

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Immunohistochemical analysis of paraffin embedded mouse testis tissue slide using IHC0345M (Mouse Phospho-CREB-1 (Ser133) Kit).