

## Mouse Calbindin Ready-To-Use IHC Kit

**Cat. No.: IHC0106M**

**Size: 50T (including a control slide)**

**Sample Type: FFPE tissue**

**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.**

### General Information

Number	Component	50T	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
4	Blocking Buffer	3 ml	RTU	2-8°C
5	Primary Antibody (Mouse Calbindin 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
8	Chromogen Component B	0.3 ml	RTU	-20°C
9	Counter Staining Reagent	5 ml	RTU	RT
10	Differentiation Reagent	6 ml	RTU	RT
11	Mounting Media	5 ml	RTU	RT
12	Control slide (Mouse kidney)	1 slide	RTU	RT
13	Datasheet	1 copy		

### Background

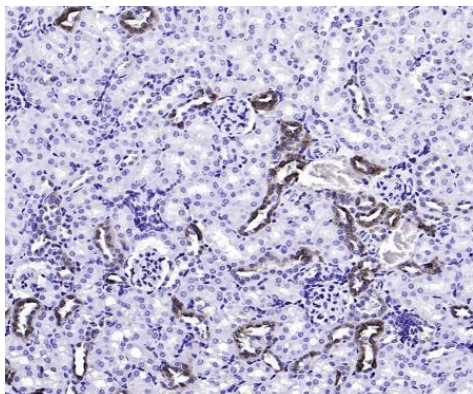
Calbindin-D-28K (also termed vitamin D-dependent calcium-binding protein, or cholecalciferin), is a highly conserved 28kDa calcium binding protein, with broad tissue distribution. It belongs, together with calmodulin, S-100, parvalbumin, troponin C and other proteins, to a family of low molecular weight calcium-binding proteins (CaBPs). These CaBPs have homologous primary structures, which contain conserved polypeptide folds of the EF-hand type for Ca<sup>2+</sup> binding. Calbindin-D-28K is found predominantly in subpopulations of central and peripheral nervous system neurons, and in certain epithelial nervous system neurons, and in certain epithelial cells involved in Ca<sup>2+</sup> transport such as distal tubular cells and cortical collecting tubules of the kidney, and in enteric neuroendocrine cells.

### Synonyms

CAB27; CALB 1; CALB; CALB1; Calbindin 1 28kDa; Calbindin-D-28K; Calbindin D28; D 28K; D28K; Vitamin D dependent calcium binding protein; Vitamin D dependent calcium binding protein avian type; Vitamin D dependent calcium binding protein avian-type; Vitamin D-dependent calcium-binding protein; CALB1\_MOUSE.

**Important Note: This product as supplied is intended for research use only, not for use in human, therapeutic or diagnostic applications.**

## Validation Data



Immunohistochemical analysis of paraffin embedded mouse kidney tissue slide using IHC0106M (Mouse Calbindin IHC Kit).

## 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 Calbindin 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.

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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 "IHC0106M, Bioss Antibodies". Citation example: "Mouse tissue sections using Mouse Calbindin IHC Kit (IHC0106M, Bioss Antibodies) were stained for Calbindin according to the manufacturer's instructions."**