

# Human MBP Ready-To-Use IHC Kit

# Cat. No.: IHC0107H

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 (Human MBP 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 (Human brain)	1 slide	RTU	RT
13	Datasheet	1 copy		

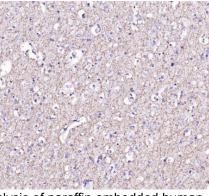
## Background

The protein encoded by the classic MBP gene is a major constituent of the myelin sheath of oligodendrocytes and Schwann cells in the nervous system. However, MBP-related transcripts are also present in the bone marrow and the immune system. These mRNAs arise from the long MBP gene (otherwise called "Golli-MBP") that contains 3 additional exons located upstream of the classic MBP exons. Alternative splicing from the Golli and the MBP transcription start sites gives rise to 2 sets of MBP-related transcripts and gene products. The Golli mRNAs contain 3 exons unique to Golli-MBP, spliced in-frame to 1 or more MBP exons. They encode hybrid proteins that have N-terminal Golli aa sequence linked to MBP aa sequence. The second family of transcripts contain only MBP exons and produce the well characterized myelin basic proteins. This complex gene structure is conserved among species suggesting that the MBP transcription unit is an integral part of the Golli transcription unit and that this arrangement is important for the function and/or regulation of these genes.

## <u>Synonyms</u>

Myelin Basic Protein; Myelin basic protien; GDB; Golli MBP; Hemopoietic MBP; HMBPR; HUGO; MBP; MGC99675; MLD; Myelin A1 Protein; Myelin Deficient; Myelin Membrane Encephalitogenic Protein; SHI; Shiverer; SP; MBP\_HUMAN.

# Validation Data



Immunohistochemical analysis of paraffin embedded human brain tissue slide using IHC0107H (Human MBP 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 **Human MBP 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×reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

<u>Please cite this product as "IHC0107H, Bioss Antibodies". Citation example: "Human Tissue sections using Human MBP IHC Kit</u> (IHC0107H, Bioss Antibodies) were stained for MBP according to the manufacturer's instructions."