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# **Human MYH1 Ready-To-Use IHC Kit**

Cat.No: IHC0208H
Applications: IHC-P
Reactivity: Human
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 (Human MYH1 Rabbit pAb)	6 ml	RTU	2-8°C
6	Secondary Antibody (Goat Anti-Rabbit IgG H&L / HRP)	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 (Human skeletal muscle)	1 slide	RTU	RT
12	Datasheet	1 сору		

Storage and

Please store components at the temperatures indicated on the individual tube labels. The  $\,$ 

Stability: kit is stable for 6 months from the date of receipt.

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 **Human MYH1 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 **Goat Anti-Rabbit IgG H&L / HRP** 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.

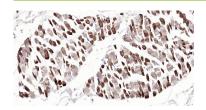
Please cite this product as "IHC0208H, Bioss Antibodies". Citation example: "Human

Tissue sections using MYH1 IHC Kit (IHC0208H, Bioss Antibodies) were stained for MYH1 according
to the manufacturer's instructions."

## Introduction:

Myosin is a protein which is known to interact with actin in muscle and non-muscle cells. It contains two identical heavy chains and four light chains. Myosin molecules consist of two major regions: tails (rods) and heads. They aggregate into filaments through the tail region and interact with actin and with ATP through the head region. Multiple forms of myosin heavy chains exist for each muscle type-skeletal, cardiac, smooth and non-muscle isomyosin forms exist in different types of skeletal muscle, depending on the physiological function of the muscle. They are designated at type I (slow twitch) and type II (fast twitch).

## **Validation Data**



Immunohistochemical analysis of paraffin embedded human skeletal muscle tissue slide using IHC0208H (Human MYH1 IHC Kit).