

## Calsequesterin Ready-To-Use IHC Kit

Cat.No: IHC0684  
Applications: **IHC-P**  
Reactivity: Human, Mouse, Rat  
Size: 50T  
Assay type: Immunohistochemistry  
Sample type: FFPE tissue  
General Information:

| Number | Component                                                                         | Size     | 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, protect from light |
| 4      | Blocking Buffer                                                                   | 3 ml     | RTU           | 2-8°C                     |
| 5      | Primary Antibody (Calsequesterin 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 (Human skeletal muscle, rat skeletal muscle, mouse skeletal muscle) | 3 slides | 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 **Calsequestrin 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.

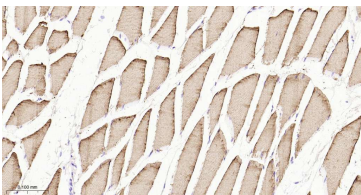
3. Please don't replace the matching reagents in this product with other manufacturers' products.
4. As DAB is a carcinogen, please take necessary precautions.
5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1× reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

***Please cite this product as "IHC0684, Bioss Antibodies". Citation example: "Tissue sections using CASQ1 IHC Kit (IHC0684, Bioss Antibodies) were stained for CASQ1 according to the manufacturer's instructions."***

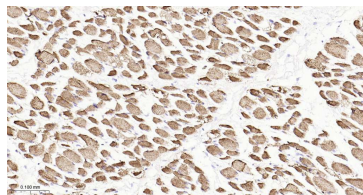
## Introduction:

The sarcoplasmic reticulum (SR) is, in part, responsible for maintaining the level of intracellular calcium in cardiac and skeletal muscle by storing and releasing calcium. Several intralumenal SR calcium binding proteins have been identified, the most prominent of these is calsequestrin. Calsequestrin is a calcium binding protein known to sequester calcium accumulated in the sarcoplasmic reticulum of muscle cells during relaxation and is found discretely localized to the junctional and corbular (terminal cisternae) SR. Calsequestrin functions to localize calcium near the junctional face of the terminal cisternae from which calcium can be released into the cytosol via the ryanodine receptor. This protein is highly acidic and has a large capacity and moderate to low affinity for calcium.

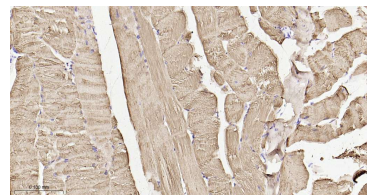
## Validation Data



Immunohistochemical analysis of paraffin embedded Rat skeletal muscle tissue slide using IHC0684 (Calsequestrin Kit).



Immunohistochemical analysis of paraffin embedded Human skeletal muscle tissue slide using IHC0684 (Calsequestrin Kit).



Immunohistochemical analysis of paraffin embedded Mouse skeletal muscle tissue slide using IHC0684 (Calsequestrin Kit).