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Phospho-SRF (Ser77) Ready-To-Use IHC Kit

Cat.No: IHC0204
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) | 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 (Phospho-SRF (Ser77) 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 colon, rat colon, mouse colon) | 3 slides | 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:

$1. \ \, \textbf{Deparaffinization And Rehydration}$

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 **Phospho-SRF (Ser77) 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.

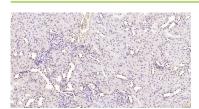
- 3. Please don't replace the matching reagents in this product with other manufacturers' products.
- 4. As DAB is a carcinogen, please take necessary precautions.
- 5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1×reagent 2) and the chromogenic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

Please cite this product as "IHC0204, Bioss Antibodies". Citation example: "Tissue sections using SRF IHC Kit (IHC0204, Bioss Antibodies) were stained for SRF according to the manufacturer's instructions."

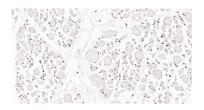
Introduction:

Serum response factor (SRF) is a transcription factor that binds the serum response element (SRE), a sequence that mediates the transient response of many cellular genes to growth stimulation. SRF-binding sites are also constitutive promotor elements in many muscle-specific promotors. SFR is required for cardiac differentiation and maturation.

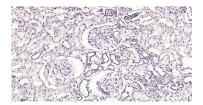
Validation Data



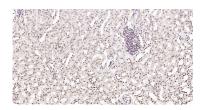
Immunohistochemical analysis of paraffin embedded rat kidney tissue slide using IHC0204 (Phospho-SRF (Ser77) IHC Kit).



Immunohistochemical analysis of paraffin embedded human skeletal muscle tissue slide using IHC0204 (Phospho-SRF (Ser77) IHC Kit).



Immunohistochemical analysis of paraffin embedded human kidney tissue slide using IHC0204 (Phospho-SRF (Ser77) IHC Kit).



Immunohistochemical analysis of paraffin embedded mouse kidney tissue slide using IHC0204 (Phospho-SRF (Ser77) IHC Kit).