SMARCA4/Brg1(SN20-03),RMab

Instruction For Use

RUO

[Product Name]

SMARCA4/Brg1(SN20-03),RMab

[Catalog No.]

CSR-0320

[Components]

Antibody Type	Rabbit Monoclonal	**Clone**	SN20-03
Isotype	IgG	**Epitope/Antigen**	SMARCA4/Brg1
Species Reactivity	Human; others not tested	**Cellular Localization**	Nucleus

[Specification]

Presentation	Dilution	Volume
Concentrate	1:100-1:250	0.1mL
Concentrate	1:100-1:250	0.2 mL
Predilute	Ready-to-Use	1 mL
Predilute	Ready-to-Use	3 mL
Predilute	Ready-to-Use	6 mL

[Intended Use]

For Research Use Only.

This antibody is intended for use in Immunohistochemical applications on formalin-fixed paraffin-embedded tissues (FFPE). The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

[Summary and Explanation]

The BRG-1 protein, encoded by the SMARCA - 4 gene on chromosome 19, is the core catalytic ATPase subunit of the SWI/SNF complex. SWI/SNFs are ATP - dependent chromatin - remodeling complexes that regulate target gene transcription. BRG-1 is vital for DNA repair, cell differentiation, and organ development, with a subunit molecular weight of around 185 kDa.

Studies show BRG-1 is involved in various cancers. Loss of BRG-1 antibody expression occurs in breast, colon, head/neck, ovarian, liver, and renal cell cancers, while overexpression is seen in breast, colorectal, prostate cancers, melanoma, and neuroblastoma. In NSCLC patients, an IHC analysis shows BRG-1 negative patients have a 0% survival rate compared to positive ones, indicating its prognostic value. Also, loss of BRG-1 protein expression is highly correlated with SCCOHT, suggesting it as a diagnostic marker for SCCOHT.

[Storage and Validity]

Store at 2~8℃. Avoid freezing. Valid for 12 months.

Maintain temperature below room temperature during transport, ensuring it does not exceed one week.

Store at 2-8°C and use within six months after opening.

[Specimen Requirements]

Tissue Preparation: Fresh biopsy or surgical samples fixed in 10% neutral buffered formalin for 8 to 24 hours. Extracted, dehydrated, and paraffin embedded according to specifications. Store wax blocks in a ventilated, dry cabinet at room temperature for 5 years.

Tissue Sectioning: Spread tissue sections (3-5 μm thick) on an adhesive slide. Tap slide rack and absorb excess water droplets with hygroscopic paper. Dry at 60℃ for 30-60 minutes or at 37℃ overnight, then cool to room temperature.

Storage: If stored at room temperature, complete the test within 7 days to maintain antigen distribution. If stored at 2-8℃, complete the test within 3 months to maintain antigen distribution.

[Protocol Recommendations]

1. Instruments and Equipment Required for Testing: Pipette, Induction cooker, Timer, Incubation box, Dye holder, Stainless steel pressure cooker, Optical microscope (40 to 400)

2. Solution Preparation:

2.1 Cleaning Solution: Wash Solution 10X Concentrate.

2.2 DAB Staining Solution: Prepare DAB Chromogen (20X) and DAB substrate buffer in a 1:20 ratio.

3. Test Temperature Conditions: Maintain a temperature of 18℃ to 25℃.

4. Test Procedure:

4.1. Manual Test Steps:

4.1.1 Dewaxing and Hydration:

- Place paraffin sections in fresh xylene, soak twice for 10 minutes each time.
- Remove excess liquid and place the sections in absolute ethanol, soak twice for 5 minutes each time.
- Remove excess liquid and place the sections in 95% ethanol for 5 minutes.
- Remove excess liquid and place the sections in 85% ethanol for 5 minutes.
- Remove excess liquid and place the sections in 70% ethanol for 5 minutes.
- Rinse with purified water for 5 minutes.

4.1.2 Antigen Repair:

- Heat the antigen retrieval solution II in a pressure cooker on an induction cooker until boiling.
- Place the dewaxed and hydrated sections on a high-temperature resistant staining rack inside the pressure cooker.
- Cover the pressure cooker and continue heating until steam is generated. After 2.5 minutes, remove the pressure cooker from the heat source and allow it to cool naturally for 5 minutes.
- Cool the pressure cooker with tap water, remove the valve, open the cover, and wait for the liquid inside the pressure cooker to cool to room temperature. Then remove the sections.
- Soak the sections in purified water twice for 3 minutes each time.
- Soak the sections in the cleaning solution once for 3 minutes.

Note: Ensure that the sections are always immersed in the repair solution during antigen repair.

4.1.3 Add the Peroxidase Block:

- Remove the cleaning solution and add 100 μL of peroxidase block to the tissue area to be tested. Incubate for 5 minutes at room temperature.
- Soak the sections twice in the cleaning solution for 5 minutes each time.

4.1.4 Add SMARCA4/Brg1 Antibody Reagent or Blank Control Reagent:

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- Remove the cleaning solution and add 100 μL of SMARCA4/Brg1 antibody reagent or blank control reagent. Incubate at room temperature for 60 minutes.
- Soak the sections twice in the cleaning solution for 5 minutes each time.

4.1.5 Add Anti-Mouse/Rabbit HRP Polymer:
- Remove the cleaning solution and add 100 μL of Anti-Mouse/Rabbit HRP Polymer dropwise. Incubate for 30 minutes.
- Soak the sections twice in the cleaning solution for 5 minutes each time.

4.1.6 Color Development:
- Remove the cleaning solution and add 100 μL of freshly prepared DAB color development solution dropwise. Incubate for 5 to 8 minutes. Staining results are typically observed within 10 minutes using a light microscope.

4.1.7 Counterstaining:
- Rinse with purified water and incubate with 100 μL of hematoxylin staining solution for 3 to 5 minutes. Wash with purified water until the blue color returns.

Note: Adjust the incubation time and intensity of hematoxylin staining solution to achieve the desired staining results. Overstaining or insufficient staining may affect the accuracy of the results.

4.1.8 Dehydration, transparent and mounting.
- Soak in 70% ethanol for 2min;
- Soak in 85% ethanol for 2min;
- 95% ethanol for 2min;
- Then absolute ethanol immersion 2 times, 2min / time;
- The final xylene transparent, neutral gum and cover glass sealing sheet.

4.2 Instrument test steps
For specific test methods, refer to the operation manual of the fully automatic IHC stainer.

[Results Interpretation]

1. Staining Results:
- Interpret results based on tissue positive control and blank control experiments: positive (+) or negative (-).
- Positive staining: Yellow or brown-yellow coloration in specific nucleus without background staining.
- Negative staining: No yellow or brown-yellow color appears in the expected cells within the tissue.

2. Presence of SMARCA4/Brg1 Antigen:
- Positive staining indicates the presence of SMARCA4/Brg1 antigen on the tissue section.
- Determine this based on positive control and blank control experiments.

3. Low Possibility of SMARCA4/Brg1 Antigen:
- Negative staining suggests a low possibility of SMARCA4/Brg1 antigen on the tissue section.
- Base this conclusion on positive control and blank control experiments.

4. Re-Testing and Quality Control:
- If both tissue positive control and blank control experiments are negative, indicating reagent failure or detection operation error, re-test the samples.
- Conduct quality control on the operation process and test results.

[Limitations]

Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized, and incubation time), optimal performance should be established through the use of positive and negative controls. Results should be interpreted by a qualified medical professional.

[Validation]

Compliance: Take the SMARCA4/Brg1 positive and negative pairs of photos according to the product instructions for immunohistochemistry testing, and locate nucleus accurately.The positive photos show yellow or brown-yellow color without background coloring, and the SMARCA4/Brg1 negative pairs of photos and the blank control are negative without coloring.

In-batch repeatability: Tissue slices from the same tissue source containing the target antigen were immunohistochemical detected with the same batch number product, and there was no significant difference in the intensity and location of tissue slice staining.

Inter-batch repeatability: Immunohistochemical tests were performed on tissue slices from the same tissue source containing the target antigen with three different batches of products, and there was no significant difference in the intensity and location of tissue slice staining.

[Troubleshooting]

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Celnovte's Technical Support at Celnovte.com.

[Precautions]

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. Always wear personal protective equipment such as a laboratory coat, goggles, and gloves when handling reagents.
3. Dispose of unused solution with copious amounts of water.
4. Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
5. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
6. Not confirmed for use on non-10% neutral formalin-fixed tissues.

[Symbols]

The following symbols may be found in this IFU or on the product labeling. Some glossary symbols may not be applicable to this product.

Symbol	Used for	Symbol	Used for
	Expiration Date		Consult instructions for use
LOT	Lot Number	RUO	For Research Use Only.
	Storage Temperature		Manufacturer
REF	Catalogue number		Date of manufacture

[Version]

Revision date: 2024-01-02

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Version: 01; Change Summary: New.

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