ihc-P SOX10 Ab
Anti-Human SOX10 (Clone R1008)

Ab: KF32040-005 and KF32040-015, 50 and 150 tissue stains*
Ab-Enh: KF49040-### (005, -010, -015, -030) 50, 100, 150, or 300 tissue stains*. * Estimated at 100µL per tissue volume

Intended Use: For In Vitro Diagnostic Use

Novodiax ihc anti-SOX10 (Sry-related HMg-Box gene 10, Clone R1008) is a rabbit monoclonal antibody intended for laboratory use to qualitatively identify by light microscopy the presence of SOX10 in sections of formalin-fixed, paraffin-embedded (FFPE) tissues using immunohistochemistry (IHC) test methods. The interpretation of any staining or its absence should be complemented by morphological studies using proper controls. These reagents have been pre-diluted and optimized for IHC when used together without further dilution.

Summary and Explanation:

ihc-P SOX10 is a monoclonal rabbit primary antibody paired with the polymerized HRP found in ihc Enhancer, a Moure-Rabbit antibody amplification reagent for IHC use on frozen and FFPE tissues. SOX10 is also called SRY-related HMG-box 10 protein, which is the transcription factor known to be crucial in the specification of the neural crest and maintenance of Schwann cells and melanocytes. It is expressed in nuclei of melanocytes, peripheral nerve sheath cells, and breast myoepithelial cells. Positive nuclear staining for SOX10 is seen in breast basal-like, unclassified triple negative, and metaplastic carcinoma. SOX10 also shows an increased specificity for soft tissue tumors of neural crest origin compared with S100. SOX10 along with other markers like MITF1 may be useful in differentiating melanoma in situ from actinic keratosis with melanocytic hyperplasia. Adding ihc Enhancer following the application of SOX10 antibody creates staining signal. A chromogen such as 3,3'-diaminobenzidine (DAB) is used to develop color at the reaction site.

Principle of Procedure:

The ready-to-use (RTU) ihc-P SOX10 antibody reagent is directly applied to pretreated tissue sections, where it binds to SOX10 antigens in tissue. Following antibody incubation, tissues are washed and a signal amplifier (ihc Enhancer) is applied. The tissues are washed again and a Working Solution (WS) of a chromogen such as ihc DAB 1:1 is applied. Polymerized HRP (pHRP) reacts with the chromogen to form a visible colored product at the site of SOX10 binding location. The specimen can be counterstained and a coverslip applied. Results are viewed and interpreted using a light microscope. Volumes are based upon 100µl antibody per tissue. This product may be used manually or on an automated IHC staining system.

Reagents Provided:

Part No.	Σ	Description
KP32040-005	50	*5ml size ihc-P SOX10 RTU antibody.*
KP32040-015	150	*15mL of ihc-P SOX10 RTU antibody*
KP49040-005	50	5ml size of ihc-P SOX10 RTU antibody plus, 5mL size of ihc Enhancer K51011-005.
KP49040-010	100	2x5mL size of ihc-P SOX10 RTU antibody plus, 2x5mL size of ihc Enhancer K51011-005.
KP49040-015	150	15ml size of ihc-P SOX10 RTU antibody plus, 15mL size of ihc-P Enhancer K51011-015
KP49040-030	300	2x15ml size ihc-P SOX10 RTU antibody plus, 2X15ml size of ihc Enhancer K51011-005.

Above volumes are estimated at 100µl of ihc antibody/ihc Enhancer per tissue

Immunogen	Clone	Species	Ig Class	Total Protein Conc.
Recombinant SOX10	R1008	Rabbit	IgG1	<8 mg/ml

SOX10 antibody is a rabbit monoclonal antibody purified from ascites. HRP is extracted from horseradish plant. The ihc Enhancer is a signal amplification reagent that is necessary for the reaction. Novodiax ihc DAB 1:1 Kit or ihc Magenta 1:1 are color forming chromogens recommended for use with the SOX10 antibody.

SOX10 Ab Components (KF32040-###):

Reagent Description	Component Part Numbers	Sizes (ml)
ihc-P SOX10	H32040-Q### (005, 015)	5, 15

SOX10 Ab-Enh Components (K49040-###, items may be shipped separately):

Reagent Description	Component Part Numbers	Sizes (ml)
ihc-P SOX10	H32040-Q### (005, 015)	5, 15
ihc Enhancer	D28020-R### (005, 015)	5, 15

Ancillary Reagents for Use with SOX10 Antibody:

Reagent Description	Part Numbers	Sizes (ml)
ihc Blocker (Intl.)	K50001-### (015)	15
ihc Blocker (USA)	K50002-### (015, 030)	15, 30
ihc Enhancer (required)	K51011-### (015, 030)	15, 30
ihc DAB 1:1 Kit	K50002-### (015, 030)	15, 30
ihc Magenta 1:1 Kit	K50011-### (015, 030)	15, 30

Materials Needed but Not Provided:

The following reagents/supplies may be required in staining but are not provided:

1. Frozen section fixative (10% NBF§ recommended)
2. Positive and negative control tissues
3. Microscope slides, positively charged (recommended)
4. Staining jars, baths or processing tools
5. ihc Wash Buffer (PBS-T)
6. Pipettor and pipet tips
7. Timer
8. Antigen retrieval buffer (when using FFPE tissues)
9. Peroxide blocker (optional)
10. Instruments used for tissue pretreatment, such as water bath, or pressure cooker or microwave oven (when using FFPE tissues)
11. Hematoxylin
12. Xylene or Xylene substitute
13. Ethanol
14. Mounting medium
15. Cover slips
16. Light microscope (40 - 400x)

§ NBF – neutral buffered formalin

Novodiax Bulk Reagent Formulations:

1. ihc Wash Buffer (PBS-T), (10 mM phosphate buffer, pH7.2, 150 mM NaCl, 0.05% Tween-20). Dilute 10X ihc Wash Buffer in DI water until 1X.
2. Antigen Retrieval Buffer (10mM Citric buffer, pH 6.0, 0.05% Tween 20).

Storage and Handling:

This reagent should be stored at 2-8°C and is suitable for use until expiration date when stored at this temperature. Do not freeze. Do not use the product after expiration date unless dating extension information is provided by Novodiax. If reagents are stored under any conditions other than those specified in the package insert, they must be validated by the user.

Warnings and Precautions:

1. Read and understand all of the Novodiax Instructions for Use (IFUs) before product use.
2. The ready-to-use ihc SOX10 antibody and ihc Enhancer signal amplification reagents are pre-diluted. Further dilution may reduce signal intensity or increase the possibility of false-negative staining. The enclosed procedures are recommendations to be used for guidance purposes. Laboratory managers should determine their own procedures and quality policies.
3. Use caution and shorten the time in solution when using intense counterstains like Gills as these stains may tend to mask antibody staining.
4. Use lab grade quality chemicals as water when preparing reagents. Users should validate performance including stability for lab prepared reagents (at 1x).
5. Take reasonable precautions when handling reagents. Use protective equipment such as disposable gloves and lab coats when handling materials. Read Safety Data Sheets (SDS) prior to use.

6. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
7. Use charged slides to secure tissue adhesion.
8. Patient specimens and all materials that come into contact with patient specimens should be handled as bio-hazardous materials and disposed of appropriately.
9. Consult local or state authorities with regard to recommended method of disposal of bio-hazardous and hazardous chemical waste materials.
10. Incubation time and temperature other than those specified may give erroneous results. The user must validate any such changes.
11. Avoid microbial contamination of reagents.
12. It is generally best to prevent slides from drying out during the staining process to avoid unwanted background staining.

Performing IHC on Paraffin Tissue Sections:

Specimen Preparation:

Paraffin Sections: Tissues routinely processed with 10% NBF are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981; Sheehan & Hrapchak, 1980). Variable results may occur as a result of prolonged fixation. Each section should be cut to the appropriate thickness (approximately 4-5 µm) and placed on a positively charged glass slide. Osseous tissues should be decalcified prior to tissue processing to facilitate tissue cutting and prevent damage to microtome blades (Kiernan, 1981; Sheehan & Hrapchak, 1980).

General Operating Notes: SOX10

1. Equilibrate all reagents to room temperature prior to use. Swirl or shake the ihc Enhancer and labeled antibody solutions before use. **Do not vortex.** Calculate the amount of chromogen WS needed (100µl per tissue) and **freshly** prepare chromogen WS. See instructions for use.
2. Gently and thoroughly wash tissues during manual wash steps. Avoid direct high velocity streams of wash that might tend to damage or cut delicate tissues.
3. Following each manual assay step, remove excess fluids on tissue slides with tissue paper. Excessive residual solution may dilute subsequent reagents, causing negative or uneven staining. Users may also utilize a PAP pen to ensure reagents stay on the desired tissues.
4. To reduce background signal, wash thoroughly following antibody and enhancer step.
5. For the tissues with high oxidase activity, blocking with H_2O_2 may be required to minimize non-specific staining.
6. The following protocol has been validated at temperatures between 21°-30°C (70°-86°F) for incubating SOX10 antibody, and ihc Enhancer and a Novodiax chromogen WS. If room temperature is less than 21°C, users may need to incubate labeled antibody for a longer period of time to achieve satisfactory staining results. Consistent results have been obtained at room temperature or using a slide warmer set to 30°C (Slide Manager 34.5°C) at the slide surface.

Paraffin Tissue Protocol:

1. Deparaffinization: Soak slides in Xylene 3 times for 5 minutes each. Next, 3 minutes each in 100%, 95% and 75% ethanol.
2. Wash slides with tap water in slide tank for two times, 2 minutes each time.
3. Antigen retrieval: Using a water bath, incubate slides in antigen retrieval buffer in a slide tank at 96°C for 30-60 minutes, then cool the slides down to room temperature for 30 minutes.
4. Rinse the slides twice with tap water, 2 minutes each time.
5. (Optional) Block tissue with H_2O_2: Soak the slides in 3% H_2O_2 in slide tank, stand for 10 minutes. Rinse the slides with tap water twice and then wash once with PBS-T in slide tank for 2 minutes. Remove excess fluid.
6. Dispense 100µl of anti-human SOX10 antibody on slides covering the entire tissue and incubate for 20-30 minutes at room temperature. Note: Place slides in a wet chamber during antibody incubation step to prevent evaporation when longer incubation times are used.
7. Rinse the slides three times with PBS-T in slide tank, 2 minutes each time. Remove excess fluid.
8. Dispense 100µl of ihc Enhancer covering the entire tissue, incubate for 3-10 minutes at room temperature.
9. Rinse the slides three times with PBS-T in slide tank, 2 minutes each time. Remove excess fluid.
10. Dispense 100µl of a chromogen WS covering the entire tissue, incubate for 3 minutes at room temperature.
11. Rinse the slides twice with DI or tap water in slide tank, 2 minutes each time.
12. Counterstaining: Add hematoxylin and incubate for 2-45 seconds depending upon the intensity of the counterstain, at room temperature.
13. Rinse twice with tap water for 2 minutes, each time.
14. Dehydration: Soak slides in the following order: 75% ethanol for 3 minutes, 95% ethanol for 3 minutes, 100% ethanol for 3 minutes and Xylene twice at 5 minutes each time.
15. Applying Coverslip: Add one drop of permanent mounting medium on both the slide and the coverslip, then apply coverslip.

Paraffin Protocol for ihc SOX10 using Standard Tissue Preparation	Time in minutes
Baking Prep Off-Line	≥ 30-60
**Optional: Xylene pretreatment*	2-4
Offline Antigen Retrieval	- - -
**Optional: Chemical Block*	5
- Wash thoroughly with ihc Wash Buffer	- - -
- Tap+Absorb to remove excess blocker	- - -
**Optional: Block with* ihc Blocker	1-3
- Tap+Absorb to remove excess blocker	- - -
Novodiax ihc Antibody reagent	10
- Wash thoroughly with ihc Wash Buffer	- - -
- Tap+Absorb to remove excess blocker	- - -
ihc Enhancer	10
- Wash thoroughly with ihc Wash Buffer	- - -
- Tap+Absorb to remove excess wash buffer	- - -
Chromogen Working Solution **(DAB / Magenta)**	**3 /3**
- Wash with ihc Wash Buffer or DI water	- - -
Hematoxylin counterstain **(Conc. Dependent)**	2-45 sec
Wash with water	- - -
Dehydrate/Mounting Medium and Coverslip	User Det.
Total	**~75 - 180**

Quality Control Procedures:

Positive and negative controls should be run simultaneously with patient specimens.

Positive Tissue Control: The recommended positive control tissues for this antibody are properly processed melanoma and skin. The staining is nuclear for melanoma cells and melanocytes in skin. One positive tissue control for each set of test conditions should be included in each staining run. Previous tissue specimens that have been frozen and freshly cut or in some cases, an individual's own tissue may be used as controls.

The tissues used for the positive control should be selected from patient specimens with characterized positive target activity that gives positive staining. Known positive tissue controls should only be utilized for monitoring the correct performance of processed tissues and test reagents, rather than as an aid in formulating a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate positive staining, results with the patient specimens should be considered invalid.

Negative Tissue Control: The same tissue used for the positive control may be used as the negative tissue control. The variety of cell types in most tissue sections offers internal negative control sites. But this should be verified by the user. The components that do not stain should demonstrate the absence of specific staining, and provide an indication of non-specific background staining. If specific staining (false positive staining) occurs in the negative tissue control sites, results with the patient specimens must be considered invalid. Breast carcinoma and lung carcinoma tissues may be used as negative tissue control.

Troubleshooting:

If an unexpected staining pattern occurs on control tissues or patient samples, consider the following:

1. *No staining*: If no staining is evident on positive control slide, please verify whether (1) chromogen WS was prepared freshly and correctly, (2) reagents were applied in the correct order, (3) that antibody was indeed added, and (4) for FFPE tissue, dewaxing and antigen retrieval were performed adequately. Perform any corrective actions required and then repeat the procedure.
2. *Low signal or faint staining*: Please check whether (1) the reagents are not expired, (2) temperature of the testing environment was at least 21°C or a 30°C slide warmer was used, (3) chromogen WS was prepared freshly and correctly, (4) excess ihc Wash solution was not left on the slide, causing subsequent reagents to be diluted, and (5) for FFPE tissue, dewaxing and antigen retrieval were performed adequately. Perform any required corrective actions and repeat the procedure. Alternatively, if using a DAB chromogen, consider using another stain, e.g. ihc Magenta 1:1 to obtain more vibrant staining. In addition, some individuals may naturally have low expression of certain antigens. In these cases, users may extend the antibody incubation times by 1-2 minutes.
3. *High background*: Possible causes include (1) insufficient washing, (2) specimens drying out, (3) prolonged chromogen incubation, (4) prolonged antibody or Enhancer incubation, and (5) specimens containing high level of endogenous peroxidase, which necessitates an additional blocking step (refer to the Staining Procedures for Paraffin Tissues). Perform any required corrective actions and repeat the procedure.

If an unexpected staining pattern is observed on control tissues or patient samples which cannot be explained by variations in laboratory procedures or a problem with the antibody is suspected, contact Novodiax Technical Support or your local distributor immediately. Within the US and Canada call 1 (888) 439-2716 ext. 2 or 1 (510) 342-3043 ext. 2.

Expected Results:

Intense color stains the tissue with a clean background if SOX10-expressed cells exist. There will be no color staining if no SOX10-expression cells exist in the tissue. Interpretation of the staining result is solely the responsibility of the user.

ihc-P SOX10 Key to Symbols

IVD	*In vitro* diagnostic medical device	SOX10 Ab	SOX10 antibody reagent
REF	Catalog Number	ihc Enhancer	pHRP Mouse/Rabbit amplification reagent
	Use by: YYYY-MM-DD		Manufacturer
	Consult Instruction for Use	Σ	Contains sufficient for < n > tests
LOT	Batch Code	2-8°C	Temperature Limitations

General Limitations:
Immunohistochemistry is a multistep diagnostic process that requires specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results. Improper fixation, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue (Nadji M, Morales AR. 1983).

The manufacturer provides these antibodies/reagents at optimal dilution for use following the provided instructions for IHC on prepared tissue sections. Any deviation from recommended test procedures may invalidate declared expected results; appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results under these circumstances.

Performance Characteristics:

The ihc-P SOX10 test performance has been determined using both frozen and FFPE tissue sections. Novodiax has conducted studies to evaluate the performance and the accompanying reagents and ancillary supplies. The antibodies and systems have been found to be sensitive and show specific binding to the antigen of interest with minimal to no binding of non-specific tissues or cells. Novodiax antibodies and accompanying reagents have shown reproducible and consistent results when used within a single run, between runs and between lots. These products have been determined to be stable for the periods of time specified on the labels either by standard real-time and/or accelerated methods. Novodiax ensures product quality by testing each lot of material and by testing materials at regular intervals and via surveillance programs.

Instructions for Use (IFU) Access:

To obtain a translation or the latest electronic version of an IFU document, visit our website at https://www.novodiax.com/literature/instructions-for-use-ifu/. Printed copies of an IFU document may be obtained by contacting Novodiax Technical Support or your local distributor.

Bibliography:

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