Q-STAIN® QSX-Dir/Enh PanCK4Ab (Clone AE1, AE3, C94, R226) Antibody Reagent

Q-STAIN® QSX-Dir PanCK4Ab QH31058-004 – 40 tests

Q-STAIN® QSX-Enh PanCK4Ab QF31058-004 – 40 tests

Intended Use: For *In Vitro* Diagnostic Use on Q-STAIN X Autostainer

Q-STAIN X (QSX™)-Dir PanCK4Ab (Clone AE1, AE3, C94, R226) and Q-STAIN X (QSX™)-Enh PanCK4Ab (Clone AE1, AE3, C94, R226) both are intended for laboratory use to qualitatively identify by light microscopy the presence of Pan-CK protein in sections of formalin-fixed, paraffin-embedded (FFPE) and/or fresh frozen tissues using immunohistochemistry (IHC) test methods. 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 and proper controls interpreted by a qualified pathologist and/or physician. This reagent has been formulated to ready-to-use concentration and optimized for IHC use without further dilution.

Summary and Explanation:

QSX (-Dir and -Enh) PanCK4Ab is ready-to-use antibody cocktail which consists of equal amounts of three mouse monoclonal antibodies clones AE1, AE3 and C94 and one rabbit monoclonal antibody clone R226. Clones AE1 and AE3, always used together as an anti-pan-cytokeratin antibody cocktail, recognize cytokeratins 1-8, 10, 14 -16 and 19; clone C94 recognizes cytokeratins 8 and 18; clone R226 specifically recognizes CK5. The classic Pan-CK antibody cocktail AE1/AE3 has been the most popular Pan-CK cocktail in the market and is known to have limited reactivity in hepatocellular carcinomas (HCCs), renal cell carcinomas (RCCs), and pulmonary small cell carcinomas, and even on FFPE tissue specimens. See Q-STAIN X User's Guide to learn more about test protocols**.**

Reagents Provided:

Component Part #	Σ	Description
H31058-Q004	40	QSX-Dir PanCK4Ab antibody reagent; intended to be used as Direct-IHC protocol
F31058-Q004	40	QSX-Enh PanCK4Ab antibody reagent; intended to be used as Enhanced-IHC protocol

Immunogen	Clone	Species	Ig Class	Total Protein Conc.
Human Pan-CK	AE1, AE3, C94, R226	Mouse/Rabbit	IgG	10 mg/mL

QSX-Dir/Enh Pan-CK 4Abs Key to Symbols

IVD	*In vitro* diagnostic medical device	QSX-Dir PanCK4Ab	QSX-Dir Pan-CK 4Abs antibody reagent
REF	Catalog Number	QSX-Enh PanCK4Ab	QSX-Enh Pan-CK 4Abs antibody reagent
	Use by: YYYY-MM-DD	GTIN	Global Trade Item Number
	Consult Instruction for Use		Manufacturer
LOT	Batch Code	Σ	Contains sufficient for < n > tests
2°C 8°C	Temperature Limitations	SN	Serial Number

Storage and Handling:

This product is suitable for use until expiration date when stored at 2-8°C. Do not freeze. Do not use the product after expiration date. If the reagent is stored under any conditions other than those specified in the package insert, they must be verified by the user.

General Operating Notes:

Equilibrate reagents to room temperature prior to use. Gently mix reagents by inverting the reagent cartridge before use. **Do not vortex.** Use care when handling Q-STAIN QSX cartridges: antibodies, Blocker, Enhancer and Chromogen reagents to avoid dispensing reagents inadvertently by applying downward pressure to the top of the cartridge. Remove reagent ventilation cap from the top and white cap from the bottom of the cartridge before placing it onto the Q-STAIN X reagent carousel.

Specimen Preparation:

Paraffin Sections: Tissues routinely processed with 10% Neutral Buffered Formalin (NBF) are suitable for use prior to paraffin embedding. Consult references.[3,4] Variable results may occur as a result of prolonged fixation. Each section should be cut to the appropriate thickness (4-6µm) and placed on a positively charged glass slide. Slides containing the tissue section may be baked for at least one hour but not exceeding 24 hours in a 58-60°C±5°C oven. Osseous tissues should be decalcified prior to tissue processing to facilitate tissue cutting and prevent damage to microtome blades.[3,4]

Frozen Tissue Sections: Frozen tissue is sectioned to the appropriate thickness (4-6µm) and placed on a positively charged glass slide. Tissues should be fixed in either 10% NBF or reagent grade acetone for 1-2 minutes immediately after sectioning. The choice of fixatives should be validated based on specific assays and tissue selection. In some cases, drying or heat fixation may help tissues adhere to the slide (~20 seconds). Other fixative solutions and fixation methods should be validated prior to use.

Warnings and Precautions:

1. Read and understand all of the Novodiax Q-STAIN X operating instructions and reagent instructions for use (IFUs) before product use.
2. It is recommended that institutions incorporating new staining protocols undergo site-specific and regulatory body-specific validation for clinical use.
3. The formulated antibody reagents and QSX Enhancer are ready to use. Further dilution may reduce signal intensity or increase false-negative staining.
4. Use care when handling QSX reagent containers and re-capping the ventilation cap. Reagent cartridges are spring loaded so accidental dispensing of reagent is possible when pressure is applied to the top of the reagent cartridge. For storage, place cartridge back into white reagent bottom cap and stow vertically.
5. To obtain best results with frozen tissues, it is desirable to process tissues as quickly as possible following excision.
6. Use Novodiax recommended counterstain. Exercise caution and shorten incubation times when using intense hematoxylin counterstains such as Gills as these stains may tend to mask antibody staining.
7. Fixation is a vital part of the protocol and fixation times may vary with the fixative chosen, tissue type, e.g. containing fat and other parameters. Generally, an acetone or NBF fixation of 1-2 minutes is recommended. Place frozen tissue sections into fixation solution shortly after sectioning.
8. **Prolonged exposure to room or freezing temperatures may alter targeted epitopes.** Avoid slides drying out during staining process to prevent non-specific background staining.
9. Use protective equipment such as disposable gloves and lab coats when handling materials. Read Safety Data Sheets (SDS) prior to use. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.

10. Patient specimens and all materials that come into contact with patient specimens should be handled as bio-hazardous materials and disposed of appropriately.
11. Consult local or state authorities with regards to recommended methods of disposal of biohazardous and hazardous chemical waste materials.
12. Use lab grade chemicals such as acetone or water when preparing reagents. Users should validate performance including stability for laboratory prepared reagents (at 1X).
13. Avoid microbial contamination of reagents and use charged slides to secure tissue adhesion.

Quality Control Procedures:

Positive and negative controls should be run simultaneously with patient specimens. It is recommended that controls be included in Q-STAIN sample runs to validate reagent performance.

Positive Tissue Control: The recommended positive control tissues for this antibody are known Pan-CK positive tissues. 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 a control.

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

Negative Tissue Control: The same tissue may be used to provide positive and negative controls. Differentiation of cells present in most tissue sections provide internal negative control sites, but this should be verified by the user. Components that do not stain should lack antibody 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 should be considered invalid.

Troubleshooting:

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

1. *No staining*: If no staining is evident on positive control slides, please verify (1) whether the Q-STAIN X chromogen is within stability claim after mixing, (2) for FFPE tissues, check to see that dewaxing and antigen retrieval were adequately performed. Take necessary corrective actions and repeat the procedure.
2. *Low signal or faint staining*: Please check whether (1) the reagents have not expired, (2) chromogen is within stability claim after mixing, (3) for FFPE tissue, that dewaxing and antigen retrieval were performed adequately, and (4) for frozen tissue, acetone or NBF are fresh and used as a fixative. Perform any required corrective actions and repeat the procedure.

If unexpected staining 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 at 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 Pan-CK-expression cells exist. There will be no color staining if no Pan-CK expression cells exist in the tissue. Interpretation of the staining result is solely the responsibility of the user.

General Limitations:

Even though the Q-STAIN X automates multistep immunohistochemistry (IHC) testing other factors can influence the success of testing. IHC testing requires training in selection of the appropriate ancillary reagents and tissue selection, fixation, and preparation of each slide. Improper fixation, freezing, thawing, drying, heating, sectioning or contamination with other tissues or fluids, may produce artifacts or false negative results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue.[5]

Performance Characteristics:

The QSX (-Dir and -Enh) PanCK4Ab test performance has been determined using both frozen and FFPE tissue sections. Novodiax has conducted studies to evaluate the performance of the antibody, 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 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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3. Kiernan JA. Histological and Histochemical Methods: Theory and Practice. New York: Pergamon Press 1981.
4. Sheehan DC and Hrapchak BB. Theory and Practice of Histotechnology. St. Louis: C.V. Mosby Co. 1980.
5. Nadji M, Morales AR. Immunoperoxidase, part I: the techniques and its pitfalls. Lab Med,1983;14:767-771.

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