FastFrozen pan CK (AE1&AE3)

Instruction For Use

RUO

[Product Name]

FastFrozen pan CK (AE1&AE3)

[Catalog No.]

FCM0200

[Components]

Antibody Type	Mouse Monoclonal	**Clone**	AE1&AE3
Positive Control	Skin, colon, prostate	**Cellular Localization**	Cytoplasm

Reagents needed but not provided: PolyStacker™ anti-Mouse Detection Kit (SD7001); Frozen Section Fixative (SD7004); Frozen Section Wash Buffer (10X) (SD7005); negative control reagent: Primary Antibody Diluent (Catalog number: SD2005); pan-CK positive control slide; xylene; ethanol (anhydrous, 95%, 85%, 70%); deionized water; neutral balsam.

[Specification]

Presentation	Dilution	Volume
Predilute	Ready-to-Use	1 mL
Predilute	Ready-to-Use	3 mL

[Intended Use]

For Research Use Only.

This reagent proves valuable in the rapid detection of pan-CK antigens within tissue sections, specifically designed for immunostaining of frozen specimens during surgical procedures. However, it is crucial to acknowledge that the test results are intended for reference purposes only and should not be relied upon as the sole diagnostic indicator. Qualified pathologists should consider various factors including clinical manifestations and other test results while interpreting the findings.

[Summary and Explanation]

Immunohistochemical staining employs antibodies with specific affinity for antigens found within tissue cells. The process involves the initial binding of a monoclonal primary antibody against pan-CK to the corresponding antigen in the tissue. Subsequently, an enzyme-labeled goat anti-mouse IgG polymer recognizes the primary antibody that has bound to the antigen. The horseradish peroxidase polymer then catalyzes the development of DAB substrate at the antigen sites.
This staining technique aids in the localization and characterization of antigens in the specimen, typically observed in the cytoplasm.

[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.

[Applicable Instrument]

Optical microscope

[Specimen Requirements]

1.Embed the specimen in OCT embedding medium inside a cryostat.
2.Cut sections at 5~8μm and mount on a charged glass slide.
3.When applying this antibody on frozen section tissues, antigen retrieval is not necessary.
4.Keep the specimen moisturized throughout the whole process.
5.To ensure accurate results, it is important to complete the staining process on the same day as the tissue fixation.

[Test Method]

Required Instruments and Equipment
Micro pipette, IHC Pap-Pen, timer, IHC wet box, staining rack, cover slide, and bright field microscope.
1. Prepare working solutions
1.1 Wash Buffer: Prepare the wash buffer from Frozen Section Wash Buffer (10X).
1.2 DAB working solution: Mix DAB Substrate (20x) and DAB Substrate Buffer in a ratio of 1:20.
2. Before experiment, make sure all the reagents are warmed up to room temperature: 20℃～25℃。
3. Staining Protocol:
3.1 Fixation: Fix the specimen in Frozen Section Fixative for 30s ~1min, rinse with diH_2O to remove OCT embedding agent.
3.2 Antigen retrieval: Antigen retrieval is not necessary when applying this antibody on frozen sections; For FFPE sections, perform HIER with pH9.0 antigen retrieval buffer.
3.3 Peroxidase Block: Rinse the slide with diH_2O. Use a IHC pap-pen to draw a circle around the tissue specimen. Add Peroxidase Blocker to the specimen and incubate for 30s. Rinse the slide with wash buffer for 30s.
3.4 Primary Antibody: Remove the wash buffer. Incubate with the corresponding FastFrozen primary antibody for 2~4mins. Rinse with wash buffer for 1min.
3.5 Detection Polymer: Remove the wash buffer, incubate with Anti-Mouse HRP-Polymer for 2~3mins. Rinse with wash buffer for 2mins.
3.6 DAB Chromogen: Remove the wash buffer, incubate with DAB working solution for 2mins. Rinse with diH_2O.
3.7 Counterstain: Incubate with hematoxylin for 10~15s. Rinse with diH_2O.
3.8 Dehydrate, clearing, and coverslip based on routine protocols.

[Positive Interpretation]

1.The staining results must be established based on the successful positive control and negative control experiments. The interpretation of the staining results is:
Positive (+) / Negative (-)
1.1 A positive staining result indicates the presence of yellow or brown-yellow staining in the cytoplasm of specific cells in the tissue section, with no background staining.
1.2 A negative staining result indicates the absence of yellow or brown-yellow staining in the expected cells within the tissue.
2.Based on the successful positive control and negative control experiments, the presence of positive staining in the test tissue section indicates the presence of pan-CK antigen in the tissue.
3.Based on the successful positive control and negative control experiments, the absence of positive staining in the

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test tissue section indicates a low likelihood of the presence of pan-CK antigen in the tissue.
4.If both the positive tissue control and negative control experiments show negative results, it indicates that the reagents may have failed or there were errors in the detection operation.

[Results Interpretation]

To interpret the staining results accurately, it is necessary to establish valid positive control and negative control outcomes:
A positive staining result is characterized by the presence of brown staining specifically within the cytoplasm of particular cells in the tissue sections, while exhibiting minimal to no background staining.
Conversely, a negative staining result is indicated by the absence of brown staining in the expected cytoplasm within the tissue.

[Limitations]

1.Proper pre-treatment of tissue specimen is crucial for precise staining results. Improper fixation, freezing, thawing, washing, drying, sectioning, or contamination may lead to false positive results, or false negative results.
2.Over or insufficient counterstain may impact the result interpretation.
3.The variability of antigen expression in diseased tissue may lead to unintended reactions during testing.
4.False positive results may be caused by non-immunological binding of proteins or substrate reaction products, as well as by red blood cells and cytochrome C.

[Validation]

1.Conformity
Perform immunohistochemical testing on pan-CK positive control slides and negative control slides according to the product instructions. The localization of the cells should be accurate, with positive staining displaying yellow or brown-yellow color, and no background staining. The pan-CK negative control slides and blank controls should be negative, with no staining.
2.Intra-batch Repeatability
Take three pan-CK positive tissue slices from the same source, and perform immunohistochemical testing using reagents from the same batch. The test results should show yellow or brown-yellow staining in the cytoplasm of the cells, indicating a positive result. The positive staining should be accurately localized, with no background staining.
3.Inter-batch Repeatability
Take three pan-CK positive tissue slices from the same source, and perform immunohistochemical testing using reagents from three different batches. The test results should show no significant difference in the staining intensity and localization of the same tissue source using different batches of reagents.

[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.

[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
CE	CE mark	EC REP	Authorized representative in the European Community

[Version]

Revision date: 2024-01-02
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