Cytokeratin HMW (34βE12),MMab

Instruction For Use

IVD CE

[Product Name]

Cytokeratin HMW (34 β E12),MMab

[Catalog No.]

CCM-0900

[Components]

Antibody Type	Mouse Monoclonal	**Clone**	34βE12
Isotype	IgG1	**Epitope/Antigen**	Cytokeratin HMW
Species Reactivity	Human; others not tested	**Cellular Localization**	Cytoplasm and cell membrane

[Specification]

Presentation	Dilution	Volume
Predilute	Ready-to-Use	1 mL
Predilute	Ready-to-Use	3 mL
Predilute	Ready-to-Use	6 mL

[Intended Use]

For In Vitro Diagnostic Use.

This antibody is intended for use in immunohistochemical (IHC) applications on formalin- fixed, paraffin- embedded (FFPE) tissues. Clinical interpretation of staining results should be supplemented by morphological analysis using appropriate controls , and must be assessed within the context of the patient's clinical history and other diagnostic findings by a qualified pathologist.

[Summary and Explanation]

The Cytokeratin HMW 34 β E12 antibody is a monoclonal antibody that reacts with high molecular weight cytokeratins. Specifically, it recognizes cytokeratins 1, 5, 10, and 14, which are found in complex epithelial tissues.

This antibody stains a wide range of squamous and ductal epithelial cells, as well as carcinomas derived from these cell types. However, it does not react with cells from simple epithelia, such as hepatocytes, pancreatic acinar cells, proximal renal tubules, or endometrial glands.

Additionally, the 34 β E12 antibody does not label nerve cells, glial cells, or mesenchymal tissues like blood vessels, as these cell types contain non-keratin intermediate filaments.

Occasional reactivity with smooth muscle cells has been observed.

Importantly, this antibody is not reactive with mesenchymal tumors, lymphomas, melanomas, neural tumors, or neuroendocrine tumors. This makes the Cytokeratin HMW 34 β E12 antibody a useful tool for distinguishing prostatic adenocarcinoma from benign prostatic hyperplasia.

In summary, the Cytokeratin HMW 34 β E12 antibody is a valuable immunohistochemical marker that specifically labels cells and tissues derived from complex epithelial lineages, including carcinomas, while sparing a variety of non-epithelial cell types and neoplasms.

[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. Bake the sections in an oven at 60℃ (±5℃) for 30 to 60 minutes. Users can adjust the temperature and time as needed according to the actual situation.

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, staining rack, Stainless steel pressure cooker, Optical microscope (40 x~400x)

2. Solution Preparation:

2.1 Wash buffer: prepared by dilution of concentrated wash buffer (10×).

2.2 DAB staining solution: prepared by mixing DAB substrate and DAB buffer at a ratio of 1:20.

3. Test Temperature Conditions: Maintain a temperature of 18℃ ~ 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 to boiling in a pressure cooker on an induction cooktop.
- Place the dewaxed and hydrated sections onto a heat-resistant staining rack , and insert the rack into 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 under running tap water, release the pressure, open the lid, and wait until the liquid in the pot cools to room temperature before removing the sections.
- Soak the sections twice in purified water for 3 minutes each time.
- Soak the sections in the wash buffer once for 3 minutes.

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

4.1.3 Add the Endogenous Peroxidase Blocking:

- Remove the wash buffer 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 wash buffer for 5 minutes each time.

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4.1.4 Add Cytokeratin HMW Antibody Reagent or Blank Control Reagent:
- Remove the wash buffer and add 100 µL of Cytokeratin HMW antibody reagent or blank control reagent. Incubate at room temperature for 60 minutes.
- Soak the sections twice in the wash buffer for 5 minutes each time.

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

4.1.6 Chromogenic Reaction
- Remove the wash buffer and add 100 µL of freshly prepared DAB color development solution dropwise. Incubate for 5 to 8 minutes. Observe the staining results under an optical microscope; generally, observation should not exceed 10 minutes.

4.1.7 Counterstaining:
- Rinse with purified water, apply 100 µL of hematoxylin staining solution, and incubate for 3–5 minutes. Rinse with purified water to return the blue color.

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 soak twice in absolute ethanol for 2 minutes each time.
- Finally, clear in xylene, and mount with neutral resin and a coverslip.

4.2 Instrument test steps
For specific test methods, refer to the operation manual of the fully automatic IHC stainer(Models: CNT300, CNT320, CNT330, CNT360).

[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 cytoplasm and cell membrane without background staining.
- Negative staining: No yellow or brown-yellow color appears in the expected cells within the tissue.

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

3. Low Possibility of Cytokeratin HMW Antigen:
- Negative staining suggests a low possibility of Cytokeratin HMW 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 Cytokeratin HMW positive and negative pairs of photos according to the product instructions for immunohistochemistry testing, and locate cytoplasm and cell membrane accurately.The positive photos show yellow or brown-yellow color without background coloring, and the Cytokeratin HMW 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 staining was performed on tissue sections from the same source containing the target antigen, using three different batches of the product. No significant differences were observed in staining intensity or localization across the tested batches.

[Troubleshooting]

Please follow the antibody-specific protocol recommendations outlined in the provided datasheet. If atypical or unexpected results are observed, 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.

[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	IVD	*In Vitro* diagnostic medical device
	Storage Temperature		Manufacturer
REF	Catalogue number		Date of manufacture
CE	CE mark	EU REP	Authorized representative in the European Community

[Version]

Revision date: 2026-01-22
Version: 03; Delete item 6 under [Precautions]: Not confirmed for use on non-10% neutral formalin-fixed tissues.

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