CE

Monoclonal Mouse Anti-Human Cytokeratin, High Molecular Weight

Clone 34βE12

Code M0630

Intended use

For in vitro diagnostic use.

Monoclonal Mouse Anti-Human Cytokeratin, High Molecular Weight (HMW), Clone 34βE12, is intended for use in immunohistochemistry (IHC). This antibody is useful for the identification of basal cells and squamous epithelium in various tissues (1-5). Results aid in the classification of prostate adenocarcinoma and classification of neoplastic tissue of epithelial origin (6-16). Differential classification is aided by the results from a panel of antibodies. 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. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

Summary and explanation

Cytokeratins are intermediate filament cytoskeletal proteins essential to development and differentiation of epithelial cells. Approximately twenty different cytokeratins have been identified and are classified and numbered according to molecular weight and isoelectric points (2). In general, most low molecular weight cytokeratins (40 – 54 kDa) are distributed in non-squamous epithelium, Moll's catalog numbers 7-8 and/or 17-20 (3). High molecular weight cytokeratins (48-67 kDa) are found in the squamous epithelium, Moll's catalog numbers 1-6 and/or 9-16 (3).

Refer to *Dako General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures for: Principle of Procedure, Materials Required, Not Supplied, Storage, Specimen Preparation, Staining Procedure, Quality Control, Troubleshooting, Interpretation of Staining, General Limitations.

Reagent provided

Monoclonal mouse antibody provided in liquid form as cell culture in 0.05 mol/L Tris-HCl, pH 7.2 and 0.015 mol/L sodium azide. This product contains stabilizing protein.

Clone: 34βE12(1) Isotype: IgG1, kappa

Mouse IgG concentration: see label on vial.

The protein concentration between lots may vary without influencing the optimal dilution. The titer of each individual lot is compared and adjusted to a reference lot to ensure a consistent immunohistochemical staining performance from lot-to-lot.

Immunogen

Solubilized immunogen keratin extracted from human stratum corneum (1).

Specificity

Anti-Cytokeratin, High Molecular Weight (Anti-CK HMW), 34βE12 has been shown to react with the 66, 57, 51 and 49 kDa proteins in Western blotting corresponding to cytokeratins 1, 5, 10 and 14 of the Moll catalog (1, 2, 4).

Precautions

1. For in vitro diagnostic use.
2. For professional users.
3. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
4. As with any product derived from biological sources, proper handling procedures should be used.
5. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
6. Unused solution should be disposed of according to local, State and Federal regulations.

Storage

Store at 2-8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

Specimen preparation

Paraffin sections: The antibody can be used for labeling paraffin-embedded tissue sections fixed in formalin. Tissue specimens should be cut into sections of approximately 4 µm.

Pre-treatment: Pre-treatment of formalin-fixed, paraffin-embedded tissue sections with heat-induced epitope retrieval (HIER) is required. Optimal results are obtained by pretreating tissues with HIER using diluted EnVision FLEX Target Retrieval Solution, High pH (50x) (Code K8004). Deparaffinization, rehydration and epitope retrieval can be performed in Dako PT Link. For details, please refer to PT Link User Guide. The following parameters should be used for PT Link: Pre-heat temperature: 65 °C; epitope retrieval temperature and time: 97 °C for 20 (±1) minutes; cool down to 65 °C. Remove slide rack from PT tank and immediately dip slides in jar/tank (e.g., PT Link Rinse Station (Code PT109)) containing diluted room temperature EnVision FLEX Wash Buffer (20x) (Code K8007). Leave slides in Wash Buffer for 1-5 minutes.

The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure. For greater adherence of tissue sections to glass slides, the use of FLEX IHC Microscope Slides (Code K8020) is recommended. After staining, the sections must be dehydrated, cleared and mounted using a permanent mounting method.

Staining procedure

These are guidelines only. Optimal conditions may vary depending on specimen type and preparation method, and should be validated individually by each laboratory. The performance of this antibody should be established by the user when utilized with other manual staining systems or automated platforms.

Dilution: The recommended dilution of Monoclonal Mouse Anti-Human Cytokeratin, High Molecular Weight, Clone 34βE12, Code M0630 is 1:100. Dilute the antibody in Dako Antibody Diluent (Code S0809). Incubate pretreated tissue sections for 20 minutes at room temperature.

Negative control: The recommended negative control reagent is Dako Negative Control, Mouse IgG1 (Code X0931), diluted to the same IgG_1 concentration as the primary antibody. Unless the stability of the diluted antibody and negative control has been established in the actual staining procedure, dilute these reagents immediately prior to use. Positive and negative controls should be run simultaneously with patient specimens.

Visualization: The recommended visualization system is EnVision FLEX, High pH (Code K8000/K8010) using a 20 minute incubation at room temperature. Follow the procedure enclosed with the selected visualization system(s).

Counterstaining: The recommended counterstain is EnVision FLEX Hematoxylin (Code K8008/K8018).

Quality control: Positive and negative control tissues as well as negative control reagent should be run simultaneously using the same protocol as the patient specimens. The positive control tissue should include squamous epithelial cells and the cells/structures should display reaction patterns as described for this tissue in the “Performance characteristics” section.

Staining interpretation

The cellular staining pattern is cytoplasmic.

Performance characteristics

Normal tissues (5):

Tissue Type (# tested)	Labeled Tissue Elements	Tissue Type (# tested)	Labeled Tissue Elements
Adrenal (3)	0/3	Ovary (3)	3/3 Surface epithelium (80-100%), cytoplasmic
Bone marrow (3)	0/3	Pancreas (3)	3/3 Pancreatic duct epithelium (50-80%), cytoplasmic
Breast (2)	2/2 Gland and duct epithelium (80%), cytoplasmic	Parathyroid (3)	0/3
Cerebellum (3)	0/3	Pituitary (3)	0/3
Cerebrum (3	0/3	Prostate (3)	3/3 Basal and ductal epithelium (90%), cytoplasmic
Cervix (2)	2/2 Surface squamous epithelium (100%), cytoplasmic	Salivary gland (3)	3/3 Excretory duct epithelium (100%), cytoplasmic
Colon (3)	3/3 Surface epithelium (10%), cytoplasmic	Skin (3)	3/3 Squamous epithelium (100%), cytoplasmic
Esophagus (3)	3/3 Squamous epithelium (100%), cytoplasmic		3/3 Sweat duct and gland epithelium (80-100%), cytoplasmic
Heart (3/3)	0/3		1/3 Sebaceous gland epithelium (100%) cytoplasmic
Kidney (3)	3/3 Renal tubule epithelium (10-30%), cytoplasmic	Small intestine (3)	3/3 Epithelium (20-50%), cytoplasmic
Liver (3)	3/3 Bile duct epithelium (50%), cytoplasmic	Spleen (3)	0/3
Lung (3)	3/3 Bronchial epithelium (10%), cytoplasmic	Stomach (3)	3/3 Gastric crypt gland epithelium (10-30%), cytoplasmic
	3/3 Alveolar epithelium (50%) cytoplasmic	Testis (3)	0/3
Mesothelial cells (2)	2/2 Mesothelium (100%), cytoplasmic	Thymus (3)	3/3 Squamous epithelium (100%), cytoplasmic

Muscle, cardiac (3)	0/3	Thyroid (3)	1/3 Follicular cells (5%), cytoplasmic
Muscle, skeletal (3)	0/3	Tonsil (3)	3/3 Squamous epithelium (100%), cytoplasmic
Nerve, peripheral (3)	0/3	Uterus (2)	2/2 Endometrial epithelium (5-80%), cytoplasmic

Abnormal tissues: Immunoreactivity with anti-CK HMW, 34βE12 antibody has been reported in squamous cell, ductal and transitional cell carcinomas including: squamous cell carcinoma of the skin, lung and nasopharynx; ductal carcinoma of the breast, pancreas, bile duct and salivary gland; transitional cell carcinomas of the bladder and nasopharynx and thymomas (6-11). Anti-CK HMW, 34βE12 has also been shown to label epithelial mesotheliomas, but was unreactive with sarcomatoid or desmoplastic mesotheliomas (12). Variable reactivity with anti-CK HMW 34βE12 has been found in adenocarcinomas of ovary, gastrointestinal tract and thyroid. Tumors reported to be largely unreactive with clone 34βE12 include adenomas of endocrine organs, carcinomas of liver (hepatocellular carcinoma), endometrium, kidney and neuroendocrine tumors (6, 7, 11). In prostate, clone 34βE12 labeled the basal cells of benign lesions including atrophy, atypical adenomatous hyperplasia and post-sclerotic hyperplasia (3, 7, 13-15). Anti-CK HMW clone 34βE12 has been reported to label tumor cells in a subset acinar adenocarcinomas(3,15). Spurious immunostaining of astrocytomas has also been reported with clone 34βE12 (7,16).

References

1. Gown AM, Vogel AM. Monoclonal antibodies to intermediate filament proteins of human cells: Unique and cross-reacting antibodies. J Cell Biol 1982; 95:414-24.
2. Moll R, Franke WW, Schiller DL. The catalog of human cytokeratins: Patterns of expression in normal epithelia, tumors and cultured cells. Cell 1982; 31:11-24.
3. Miettinen M. Keratin immunohistochemistry: update of applications and pitfalls. Pathol Ann 1993; 28:113-43.
4. Gown AM, Vogel AM. Monoclonal antibodies to human intermediate filament proteins II. Distribution of filaments in normal human tissues. Amer J Pathol 1984; 114:309-21.
5. IR051(IHC003-D09346).
6. Gown AM, Vogel AM. Monoclonal antibodies to human intermediate filament proteins. III. Analysis of tumors. Am J Clin Pathol.1985 Oct;84(4):413-24.
7. Diagnostic immunohistochemistry: theranostic and genomic applications. Dabbs, David J. 3rd ed. Philadelphia, PA : Saunders /Elsevier, 2010.
8. Hurlimann J, Gardiol D. Immunohistochemistry in the differential diagnosis of liver carcinomas. Amer J Surg Pathol 1991; 15:280-8.
9. Dairkee SH, Puett L, Hackett AJ. Expression of basal and luminal epithelium-specific keratins in normal, benign and malignant breast tissue. J Nat Can Inst 1988; 80:691-5.
10. Thike AA, Cheok PY, Jara-Lazaro AR, Tan B, Tan P, Tan PH. Triple-negative breast cancer: clinicopathological characteristics and relationship with basal-like breast cancer. Mod Pathol 2010;23(1):123-33.
11. Gown AM, Vogel AM. Anti-intermediate fillament monoclonal antibodies; tissue-specific tools in tumor diagnosis. Surv Synth Pathol Res. 1984;3:369-385.
12. Bolen JW, Hammar SP, McNutt MA. Reactive and neoplastic serosal tissue: a light-microscopic, ultrastructural, and immunocytochemical study. Amer J Surg Pathol 1986; 10:34-47.
13. Varma M, Amin MB, Linden MD, Zarbo RJ. Discriminant staining pattern of small glandular and preneoplastic lesions of the prostate using high molecular weight cytokeratin antibody—A study of 301 consecutive needle biopsies. Mod Pathol 1997; 10:93A
14. O'Malley FP, Grignon DJ, Shum DT. Usefulness of immunoperoxidase staining with high-molecular-weight cytokeratin in the differential diagnosis of small-acinar lesions of the prostate gland. Virch Arch Pathol Anat 1990; 417:191-6.
15. Brimo F, Epstein JI. Immunohistochemical pitfalls in prostate pathology. Hum Pathol 2012;43:313-24
16. Bacchi CA, Zarbo RJ, Jiang JJ, Gown AM. Do glioma cells express cytokeratin? Appl Immunohistochem 1995; 3:45-53.

Explanation of symbols

REF	Catalogue number		Temperature limitation	IVD	In vitro diagnostic medical device
	Manufacturer	LOT	Batch code		Contains sufficient for <n> tests
	Use by		Consult instructions for use	EC REP	Authorized representative in the European Community

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TX02438/01

Revision 2020.07