Monoclonal Mouse Anti-Human Cytokeratin Clones AE1/AE3

Code M3515

Intended use
For in vitro diagnostic use.

Monoclonal Mouse Anti-Human Cytokeratin, Clones AE1/AE3 is intended for use in immunohistochemistry (IHC). The antibody identifies two epitopes present on a majority of epithelial cytokeratins in formalin-fixed, paraffin-embedded tissue. Results aid in the classification of normal and neoplastic tissue as epithelial in origin[1-3]. Differential classification of tumors 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 a family of water-soluble proteins with molecular weights between 40-70 kDa that form the cytoskeleton of epithelial cells. At least 19 different cytokeratins have been identified and can be divided into two subfamilies. Subfamily A comprises relatively acidic cytokeratins (with a pI under 5.5) whereas members of subfamily B have a relatively basic pI of 6 or over.

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 tissue culture supernatant in 0.05 mol/L Tris-HCl, pH 7.2 and 0.015 mol/L sodium azide. This product contains stabilizing protein.

Clones: AE1/AE3[1,2] 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
Human epidermal callus[1]

Specificity
AE1/AE3 is a cocktail of two monoclonal antibodies that were obtained by immunizing mice with human callus keratins.[2] AE1/AE3 has been shown to identify the majority of human cytokeratins and thus may be used as a tool for the positive IHC identification of cells of simple and stratified epithelial origin.[1,2,4] Antibody AE1 immunoreacts with an antigenic determinant present on most of the subfamily A cytokeratins, including cytokeratins with Moll's designation[4] 10, 13, 14, 15 16 and 19 (MWs of 56.5, 54', 50, 50', 48 and 40 kDa, respectively) but not on Nos. 12, 17 and 18 (55, 47 and 45 kDa).[4] Antibody AE3 reacts with an antigenic determinant shared by the subfamily B cytokeratins including Nos. 1 and 2, 3, 4, 5, 6, 7 and 8 (MWs of 65, 67, 64, 59, 58, 56, 54 and 52 kDa, respectively).[5]

Materials required, but not supplied
Refer to *Dako General Instructions for Immunohistochemical Staining* and/or the detection system instructions.

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, NaN_3 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 reagents 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 labelling formalin-fixed, paraffin-embedded tissue sections fixed in formalin. Tissue specimens should be cut into sections of approximately 4 µm.

Pretreatment: Pre-treatment of deparaffinized tissues with proteolytic enzymes or heat-induced epitope retrieval is recommended. For heat-induced epitope retrieval optimal results are obtained with EnVision FLEX Target Retrieval Solution, High pH (50x) (Code K8004). Epitope retrieval can be performed in Dako PT Link. For details, please refer to PT Link User Guide.

As an alternative to heat induced epitope retrieval, enzyme pretreatment can be used. The following enzymes can be used for pretreatment of formalin-fixed, paraffin-embedded tissues: Proteinase K, RTU (Code S3020), Pepsin (Code S3002), or Proteolytic Enzyme, RTU (Code S3007). Rinse thoroughly with distilled water and continue with the staining procedure of the detection system instructions.

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: M3515 may be used at a dilution of 1:50 when performing IHC using the EnVision FLEX, EnVision or LSAB2 detection systems. Follow the procedure enclosed with the selected visualization system(s).

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.

Product specific limitations
1. Extrafollicular reticulum cells of lymph nodes, tonsil and spleen have been shown to react with antibodies to cytokeratin 8.[6]
2. The presence of cytokeratin 19 and possibly 8 has been confirmed in smooth muscle cells of the uterus.[7]
3. Rare melanomas[8] and leiomyosarcomas[7] may stain positive. This finding is usually more pronounced on frozen tissue rather than formalin-fixed tissue.[9]
4. False-positive staining of glial cells in tumors has been reported on formalin-fixed, paraffin-embedded tissue when proteolytic pretreatment was employed. It was shown by immunocytochemical and biochemical methods that these cells and tumors do not express cytokeratins.[10]
5. Pinkus et al.[11] stressed the importance of proteolytic digestion of formalin-fixed tissues to be stained with AE1/AE3. Photos of stained tissues depicted include the results when digestion with trypsin II was omitted, other trypsin enzymes were used and/or when suboptimal digestion techniques were applied. In the latter cases, only 2/12 epithelial neoplasms of various types exhibited optimal staining for cytokeratin. By reviewing conflicting cytokeratin immunoreactivities in earlier publications and comparing the same with their own, Pinkus et al.[11] considered many previously false-negative cases attributable to one or several of these short-comings.
6. From a comparison of AE1/AE3 with an anti-epithelial membrane antigen (EMA) antibody in an IHC study of 87 neoplasms, including 48 adenocarcinomas of various types, Pinkus et al.[12] concluded that the cytokeratin proteins in proteolytically treated formalin-fixed tissues and as stained by AE1/AE3 were more reliable markers in 33% of the cases of epithelial derived neoplasms than anti-EMA. However, because EMA was labeled and AE1/AE3 unlabeled in 9% of the cases, it was recommended that AE1/AE3 and anti-EMA be used as complementary reagents.
7. Although no false-positive staining was reported by Listrom and Dalton,[13] faint cytoplasmic staining was observed in 2/2 plasmacytomas, 2/4 melanomas and 2/7 lymphomas and considered to be the result of nonspecific background staining.

Staining interpretation
The cellular staining pattern for AE1/AE3 is cytoplasmic.

Performance characteristics
Normal tissues: Testing of 30 different normal tissues demonstrated staining in the cytoplasm of squamous and columnar epithelium of the cervix, colon, esophagus, skin, small intestine, stomach and tonsil. Other tissues that stained included glandular tissue (mammary, parathyroid, prostate sweat and thyroid), astrocyte, white matter of the cerebellum, glial filaments of the cerebrum, distal tubule and Bowman's capsule of the kidney, bile duct, pneumocytes, bronchi, mesothelium, interlobular duct of the pancreas, anterior pituitary cell, interlobular duct and acinar cells of the salivary gland, reticular cells and Hassall's bodies of the thymus, and endometrium and smooth muscle of the uterus.[14] No staining was noted for adrenal, bone marrow, heart, pericardium, peripheral nerve, skeletal muscle, spleen and testis.

AE1/AE3 reacts with keratinized (56.5/65-67) and corneal (55/64) epidermis, stratified squamous epithelia of internal organs (51/59), stratified epithelia (50/58), hyperproliferative keratinocytes (48/56) and simple epithelia (45/52 and 46/54). The 40 kDa keratin is present in most epithelia except adult epidermis.[3,4]

Abnormal tissues: In pathological tissues, Listrom and Dalton[13] tested clones AE1/AE3 on over 60 poorly differentiated epithelial neoplasms, lymphomas, melanomas and sarcomas. Except for staining of only 2/6 cases of small cell carcinoma and 3/5 transitional cell carcinoma, the study found all of 34 epithelial neoplasms to stain. When labeled, AE1/AE3 stained transitional cell carcinomas only weakly and staining of the tumor cells was either diffusely cytoplasmic or perinuclear. Montag et al.[15] found AE1/AE3 to be a sensitive reagent for the classification of diffuse malignant mesothelioma of the sarcomatoid (spindle-cell) type (positive in 30/30 cases). When compared with anti-EMA in a study of 87 neoplasms, including 48 adenocarcinomas of various types, Pinkus et al.[12] found AE1/AE3 to stain 33% of the cases more reliably than the anti-EMA.

Although 3/3 cases of chondroid chordoma and 1/8 cases of lymphoma were reactive with anti-AE1/AE3, no staining was observed among 25 non-epithelial neoplasms including 4 cases each of melanoma and glioblastoma.[13]

References

1. Tseng SCG, Jarvinen MJ, Nelson WG, Huang JW, Woodcock-Mitchell J, Sun TTl. Correlation of specific keratins with different types of epithelial differentiation: Monoclonal antibody studies. Cell 1982;30(2):361-72
2. Woodcock-Mitchell J, Eichner R, Nelson WG, Sun TT. Immunolocalization of keratin polypeptides in human epidermis using monoclonal antibodies. J Cell Biol 1982;95(2 Pt 1):580-8
3. Moll R, Franke WW, Schiller Dl. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 1982;31(1):11-24
4. Sun T-T, Eichner R, Schermer A, Cooper D, Nelson WG, Weiss RA. Classification, expression and possible mechanisms of evolution of mammalian epithelial keratins: A unifying model. In: Levine A, Topp W, Vande Woude G, Watson JD (eds.). Cancer cells 1 the transformed phenotype. New York: Cold Spring Harbor Laboratory 1984
5. Eichner R, Bonitz P, Sun T-T. Classification of epidermal keratins according to their immunoreactivity, isoelectric point and mode of expression. J Cell Biol 1984;98(4):1388-96
6. Franke WW, Moll R. Cytoskeletal components of lymphoid organs. Differentiation 1987;36(2):145-63
7. Gown AM, Boyd HC, Chang Y, Ferguson M, Reichler B, Tippens D. Smooth muscle cells can express cytokeratins of “simple” epithelium. Amer J Pathol 1988;132(2):223-32
8. Zarbo RJ, Gown AM, Nagle RB, Visscher DW, Crissman JD. Anomalous cytokeratin expression in malignant melanoma: One- and two-dimensional western blot analysis and immunohistochemical survey of 100 melanomas. Mod Pathol 1990;3(4):494-501
9. Brown DC, Theaker JM, Banks PM, Gatter KC, Mason DY. Cytokeratin expression in smooth muscle and smooth muscle tumors. Histopathol 1987;11(5):477-86
10. Bacchi CA, Zarbo RJ, Jiang JJ, Gown AM. Do glioma cells express cytokeratin? Appl Immunohistochem 1995;3(1):45-53
11. Pinkus GS, O’Connor EM, Etheridge CL, Corson JM. Optimal immunoreactivity of keratin proteins in formalin-fixed, paraffin-embedded tissue requires preliminary trypsinization. J Histochem Cytochem 1985(5);33:465-73
12. Pinkus GS, Etheridge CL, O’Connor EM. Are keratin proteins a better tumor marker than epithelial membrane antigen? Amer J Clin Pathol 1986;85(3):269-77
13. Listrom MB, Dalton LW. Comparison of keratin monoclonal antibodies MAK-6, AE1:AE3 and CAM-5.2. Amer J Clin Pathol 1987;88(3):297-301
14. Report of Normal Tissue Immunohistochemical testing using DAKO Mouse Anti-Human Cytokeratin, Clone AE1/AE3. DAKO Corp June 1998
15. Montag AG, Pinkus GS, Corson JM. Keratin protein immunoreactivity of sarcomatoid and mixed types of diffuse malignant mesothelioma: An immunoperoxidase study of 30 cases. Hum Pathol 1988;19(3):336-42

Explanation of symbols

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

Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
Santa Clara, CA 95051
United States

Tel. +44 161 492 7050
www.agilent.com

TX02438/01

Revision 2020.07