PEVIVA

The dead cells still count!™

Store at 2–8 °C

For life science research only. Not for use in diagnostic procedures. FOR *IN VITRO* USE ONLY.

M6 Keratin 18

200 tests (Peviva Prod. No. 10650)
Mouse monoclonal antibody (Clone M6)
For the detection of keratin 18

1. Product Description

Name: M6 Keratin 18 monoclonal antibody
Clone: M6
Isotype: Mouse IgG2a
Immunogen: Keratin 18 (K18) fragments purified from human carcinomas.
Epitope: Internal on K18 fragment aa284-396.

1.1 Formulation

Clear solution. 10 µg of M6 Keratin 18 antibody provided in 100 µl PBS containing 0.1 % BSA, PEG, sucrose and 0.09 % sodium azide.

1.2 Specificity

M6 Keratin 18 antibody recognises an internal formalin-resistant epitope on human, monkey, mouse, rat and dog K18 cytoskeletal protein.

1.3 Recommended applications

- Immunocytochemistry (ICC)
- Flow cytometry (FACS)
- Western blot (WB)
- Immunoprecipitation (IP)
- Immunohistochemistry (IHC) including formalin-fixed and paraffin-embedded tissue (FFPE) and cryostat/frozen sections (FS).

1.4 Working solution

M6 Keratin 18 antibody is provided in a convenient stock solution. Use a dilution 1:200 in Incubation Buffer (final concentration 0.5 µg/ml).

1.5 Storage and stability

The M6 Keratin 18 antibody is provided in a ready-to-use format and is stable at 2–8 °C through the expiration date printed on the label. Alternatively, it can be stored in aliquots at -20 °C. The antibody is shipped at ambient temperature.

Note: Repeated freezing and thawing should be avoided.

1.6 Quality control

The M6 Keratin 18 antibody is function tested using a cellular model: HeLa cells analysed by immunocytochemistry and flow cytometry. FFPE tissue sections obtained from human prostate cancer analysed by immunohistochemistry.

M6 Keratin 18 Antibody – Overview

Benefits	Features
Exceptional broad range of applications, including FFPE.	Suitable for WB, IP, ICC, IHC (PS/FS) , FC on endogenous native K18.
Specificity for simple epithelial (i.e carcinoma) cells and tissue.	Expression pattern of K18 is restricted to cells of epithelial origin. Lymphoid, fibroblast and neuronal cells are not detected by M6 Keratin 18 antibody.

2. Background Information

2.1 Keratins

In eukaryotic cells, the cytoskeleton is composed of three different types of morphologically distinct filamentous structures: microfilaments, intermediate filaments (IF), and microtubules. The integrated cytoskeletal network formed by the three filament systems is responsible for the mechanical integrity of the cell and is a critical participant in several cellular processes, such as cell division, motility, and cell–cell contact.

For the intermediate filament (IF) protein family, a classification system divided into several groups has been implemented that is based on their characteristics such as sequence similarities and patterns of expression. Intermediate filament types I and II constitute the keratins (acidic and basic proteins, respectively), while the type III IF group includes desmin, vimentin, and glial fibrillary acidic proteins. Type IV includes the neurofilament proteins (NF-L, NF-M, and NF-H) and internexin, while type V proteins are known as nuclear lamins, exclusive to the cell nuclei. The remaining IF proteins, sometimes called type VI, include filensin and phakinin.

The epithelial keratins (IF types I and II) are conserved phylogenetically and are closely related, biochemically and immunologically. Keratins 1–8 constitute the type II group (53–68 kDa, neutral to basic protein components), while keratins 9–20 constitute the type I group (40–56 kDa, acidic proteins). This dual nature of the keratins is functionally important as the keratin proteins assemble into obligate noncovalent heterodimers containing one keratin protein of type I and one keratin protein of type II (such as keratins 8 and 18) in stochiometric amounts [1–3].

2.2 Keratins as biomarkers in epithelial cancer

Keratins, belonging to the intermediate filament (IF) protein family, are particularly useful tools in oncology diagnostics. More than 20 different keratins have been identified, of which keratins 8, 18, and 19 are the most abundant in simple epithelial cells. Upon release from dying cells, keratins provide useful markers for epithelial malignancies, distinctly reflecting ongoing cellular turnover.

Certain keratins, the most prominent example being keratin 18, are substrates for lethal caspase activation and the subsequent release of defined protein fragments occurs during apoptotic cell death. The clinical value of determining different soluble keratin protein fragments in body fluids lies in the early detection of recurrence and the fast assessment of the efficacy of therapy response in epithelial cell carcinomas [4-7].

2.3 Keratins during apoptosis and necrosis

The morphological features of apoptosis such as cell shrinkage, nuclear fragmentation, and apoptotic body formation arise from the cleavage of specific cellular (“death”) substrates by caspases.

Specific to apoptosis, one consequence of this cleavage is the exposure of a C-terminal neo-epitope (at cleavage site Asp396) in keratin 18 (designated “M30”), which is not present in the keratin 8/18 complex in vital or necrotic epithelial cells. The biological significance of keratin cleavage during apoptosis is not fully understood, although it has been suggested that caspase cleavage of the keratin proteins is likely to facilitate the formation of apoptotic bodies and amplify the apoptotic signal.

In vitro experiments have shown that cellular release of keratin 18 fragments into the extracellular space occurs as a consequence of caspase cleavage and thereby can serve as markers of apoptosis, using the M30 CytoDEATH™ antibody or the M30 Apoptosense® ELISA [8–12].

3. Procedures and Materials required

3.1 Procedure for immunohistochemistry

3.1.1 Introduction

The following procedure describes the detection of K18 with M6 Keratin 18 antibody in immunohistochemistry for paraffin-embedded tissue in a three step method for maximal sensitivity.

If using other detection methods or sample material, conditions may vary and may have to be adapted.

Preparation of working solutions

The following table lists the working solutions needed to perform the immunohistochemistry staining procedure.

Working solution	Composition	Stability Storage
Washing Buffer	PBS containing 0.05 % Tween® 20	4 weeks at 2–8 °C
Incubation Buffer	PBS containing 1 % BSA	4 weeks at 2–8 °C
Antigen retrieval	Tris 10 mM, pH 9.0 0.05 % Tween® 20	4 weeks at 2–8 °C

Preparation of antibody working solution

Dilute M6 Keratin 18 antibody stock solution 1:200 in Incubation Buffer (final concentration 0.5 µg/ml).

Note: The antibody solutions should be free of precipitate; if necessary, centrifuge vial at high speed prior to use.

Preparation of sample material

Before starting the immunohistochemical protocol, dewax paraffin-embedded tissue sections as described in the following table.

Step	Action
1	Place paraffin-embedded sections into an incubator at 37 °C over night to air-dry.
2	For deparaffinisation of FFPE tissue slides, process as follows at 15–25 °C for 3 min each: 3 × in xylol 2 × in ethanol (96 %) 1 × in ethanol (80 %) 1 × in methanol freshly prepared with H_2O_2 (3 %) for 10 min.
3	Rinse 3 × for 10 min in PBS containing 0.05 % Tween® 20.

Note: The sections should not be allowed to dry during this procedure.

3.1.2 Immunohistochemistry protocol

Note: For optimal results it is recommended to follow the method for antigen retrieval described in the table below.

Step	Action
1	Autoclave tissue section slides 10 min at 121 °C. Let cool down in antigen retrieval buffer 30 min at 15–25 °C. Place in PBS plus 0.05 % Tween® 20 for 10 min at 15–25 °C.
2	Incubate with blocking solution i.e. PBS containing 5 % normal serum (i.e. from goat, if secondary antibody from goat).
3	Remove blocking solution and add 100 µl M6 Keratin 18 antibody working solution for 30 min at 15–25 °C in a humid chamber.
4	Wash slides in Washing Buffer (use 3 separate jars and dip 3 × into each jar).
5	Cover the section with 100 µl of anti-mouse-IgG biotin according to your preferred optimised procedure for the reagent from your selected supplier. Incubate for 30 min at 37 °C in a humid chamber.
6	Wash slides in Washing Buffer (use 3 separate jars and dip 3 × into each jar).
7	Cover the section with 100 µl of streptavidin-POD according to your established optimised procedure for the reagent from your selected supplier (i.e. ABC reagent from Vectorlabs). Incubate for 30 min at 15–25 °C in a humid chamber.
8	Wash slides In Washing Buffer (use 3 separate jars and dip 3 × into each jar). Dehydrate depending on used substrate, i.e. for DAB.
9	Incubate slides in a freshly prepared substrate solution (i.e. AEC) at 15–25 °C until a clearly visible colour develops (1–5 min). A negative control should not show any development of colour during the incubation period.
10	Stop the reaction by extensive rinsing in double distilled water.
11	Subsequently, counterstain the preparation and mount sections i.e. in Aquatex for AEC, or in Vectamount for DAB.

3.1.3 Immunohistochemistry results

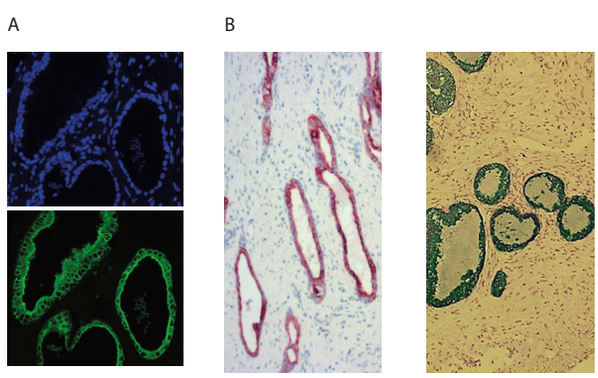

Figure: **A.** Detection of K18 in frozen section from human prostate tissue showing confined staining for K18 in epithelial cells using M6 Keratin 18 (lower panel) and nuclear counterstain with DAPI (upper panel). Secondary detection with anti mouse IgG-ALEXA-488.

B. Formalin-fixed and paraffin-embedded (left panel) or frozen (right panel) sections from human prostate tissue show confined epithelial staining for K18 with M6 Keratin 18. Secondary detection with anti-mouse IgG biotin, streptavidin-POD and AEC or Histogreen as substrate, respectively.

Images by courtesy of Claudia Strele, Tissuegnostics, Vienna, Austria.

3.2 Procedure for immunofluorescence and flow cytometry

3.2.1 Introduction

The following procedure describes the detection of K18 with M6 Keratin 18 antibody in immunofluorescence and flow cytometry. If using other detection methods or sample material, conditions may vary and may have to be adapted.

Preparation of antibody working solution

Dilute M6 Keratin 18 antibody stock solution 1:200 in Incubation Buffer (final concentration 0.5 µg/ml).

Note: The antibody solutions should be free of precipitate; if necessary, centrifuge vial at high speed prior to use.

3.2.2 Immunofluorescence and flow cytometry protocol

Step	Action
1	Wash cells in PBS.
2	Fix cells in ice-cold pure methanol at -20 °C for 30 min.
3	Wash cells with Washing Buffer twice.
4	Remove Washing Buffer.
5	Incubate with 100 µl M6 Keratin 18 antibody working solution for 30 min at 15–25 °C.
6	Wash cells with Washing Buffer twice.
7	Examine the cells on a slide under the fluorescence microscope, or dilute cells in 0.5 ml PBS and store samples in the dark until analysis by flow cytornetry.

3.2.3 Immunofluorescence and flow cytometry results

A B

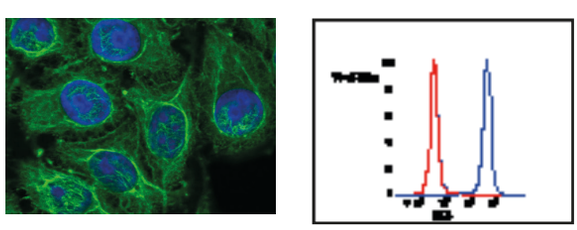

Figure: **A.** HeLa (human cervical cancer) cells were fixed in methanol and stained with M6 Keratin 18 antibody followed with goat anti-mouse IgG $F(ab)_2$-FITC or anti-mouse-IgG-ALEXA-488 before being analysed by fluorescence microscopy or **B.** by flow cytometry, respectively (blue line, compared to staining with isotype control antibody red line).

4. Appendix

K18 positive cell lines and tissues successfully analysed with M6 Keratin 18 antibody:

Human epithelial cell lines:

Breast cancer:	MDA-MB-231, MCF-7, HBL100, HT29, T47D
Colon cancer:	WiDr, HCT116, SW480, SW620
Prostate cancer:	PC3, LNCaP, DU145
Cervical cancer:	HeLa
Liver cancer:	HepG2
Lung cancer:	A431
Head & neck cancer:	SCC9, SCC25

Human epithelial tissues:

Breast, lung, liver, prostate, colon, pancreas, intestine, kidney, salivary gland, trophoblast, endometrium, bladder, oral epithelium.

5. References

1. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. (1982) The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 31:11–24.

2. Fuchs E, Weber K. (1994) Intermediate filaments: structure, dynamics function and disease. Ann Rev Biochem. 63:345–82.

3. Kirfel J, Magin TM, Reichelt J. (2003) Keratins: a structural scaffold with emerging functions. Cell Mol Life Sci. 60:56–71.

4. Chu PG, Weiss LM. (2002) Keratin expression in human tissues and neoplasms. Histopathol. 40:403–39.

5. Lane EB, Alexander CM. (1990) Use of keratin antibodies in tumor diagnosis. Cancer Biol. 1:2707–14.

6. Barak V, Goike H, Panaretakis KW, *et al.* (2004) Clinical utility of cytokeratins as tumor markers. Clin Biochem. 37:529-540.

7. Rydlander L, Ziegler E, Bergman T, *et al.* (1996) Molecular characterization of a tissue-polypeptide-specific-antigen epitope and its relationship to human cytokeratin 18. Eur J Biochem. 241:309–14.

8. Kramer G, Erdal H, Mertens H, Nap M, *et al.*(2004) Differentiation between cell death modes using measurements of different soluble forms of extracellular cytokeratin 18. Cancer Res. 64: 1751-6.

9. Leers MP, Kolgen W, Bjorklund V, *et al.* (1999) Immunocytochemical detection and mapping of a cytokeratin 18 neo-epitope exposed during early apoptosis. J Pathol. 187:567–72.

10. Caulin C, Salvesen GS, Oshima RG. (1997) Caspase cleavage of keratin 18 and reorganization of intermediate filaments during epithelial cell apoptosis. J Biol Chem. 138:1379–94.

11. Dinsdale D, Lee JC, Dewson G, *et al.* (2004) Intermediate filaments control the intracellular distribution of caspases during apoptosis. Am J Pathol. 164:395-407.

12. Sheard MA, Vojtesek B, Simickova M, Valik D. (2002) Release of cytokeratin-18 and -19 fragments (TPS and CYFRA 21-1) into the extracellular space during apoptosis. J Cell Biochem. 85:670–7.

For further, up-to-date information and to order, please visit ***www.vlvbio.com****.*

VLVbio

VLVbio AB, Hästholmsvägen 32, 131 30 Nacka, Sweden
Website: www.vlvbio.com • E-mail: info@vlvbio.com
Phone: +46 8 122 053 00