Cytokeratin 20 (CK20)  
Concentrated and Prediluted Monoclonal Antibody

901-062-041919

**Intended Use:**
For In Vitro Diagnostic Use

Cytokeratin 20 (CK20) [Ks20.8] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of cytokeratin 20 (CK20) protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. 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.

**Summary and Explanation:**
Cytokeratin 20 is a 46 kDa intermediate filament protein that has been identified with expression primarily restricted to gastric and intestinal epithelium, urothelium, and Merkel cells. Cytokeratin 20 has been shown as a unique type I keratin that is expressed in adenocarcinomas of the colon, stomach, pancreas and bile system. It is also expressed in mucinous ovarian tumors, transitional cell carcinomas of the urinary tract, and Merkel cell carcinomas. CK20 is essentially non-reactive in squamous cell carcinomas and adenocarcinomas of the breast, lung, and endometrium, as well as non-mucinous tumors of the ovary and small cell carcinomas. Cytokeratin 20 is often used in conjunction with CK7 and other antibodies in distinguishing colon carcinomas (CK20+) from ovarian, pulmonary, and breast carcinomas.

**Principle of Procedure:**
Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

**Source:** Mouse monoclonal

**Species Reactivity:** Human; others not tested

**Clone:** Ks20.8

**Isotype:** IgG2a

**Protein Concentration:** Call for lot specific Ig concentration.

**Epitope/Antigen:** CK20

**Cellular Localization:** Cytoplasmic

**Positive Tissue Control:** Colon carcinoma

**Known Applications:**
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Supplied As:** Buffer with protein carrier and preservative

**Storage and Stability:**
Store at 2°C to 8°C. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

**Protocol Recommendations (VALENT Automated Slide Staining Platform):**
VLTM062 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

- **Deparaffinization:** Deparaffinize for 8 minutes with Val DePar.
- **Pretreatment:** Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X (use at 1X).
- **Peroxidase Block:** Block for 5 minutes with Val Peroxidase Block.

**Protocol Recommendations (VALENT Automated Slide Staining Platform) Cont’d:**

- **Protein Block (Optional):** Incubate for 10-20 minutes with Val Background Block.
- **Primary Antibody:** Incubate for 20 minutes.
- **Secondary:** Incubate for 10 minutes with Val Mouse Secondary.
- **Linker:** Incubate for 10 minutes with Val Universal Linker.
- **Polymer:** Incubate for 10 minutes with Val Universal Polymer.
- **Chromogen:** Incubate for 5 minutes with Val DAB.
- **Counterstain:** Counterstain for 5 minutes with Val Hematoxylin.

**Protocol Recommendations (intelliPATH FLX® and manual use):**

- **Peroxide Block:** Block for 5 minutes with Peroxidized 1.
- **Pretreatment:** Perform heat retrieval using Diva or Reveal Decloaker. Refer to the Diva or Reveal Decloaker data sheet for specific instructions.
- **Protein Block (Optional):** Incubate for 5-10 minutes at RT with Background Punisher.
- **Primary Antibody:** Incubate for 30 minutes at RT.
- **Probe:** Incubate for 10 minutes at RT with a secondary probe.
- **Polymer:** Incubate for 10-20 minutes at RT with a tertiary polymer.
- **Chromogen:** Incubate for 5 minutes at RT with Biocare’s DAB - OR - Incubate for 5-7 minutes at RT with Warp Red.
- **Counterstain:** Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha’s Bluing Solution for 1 minute. Rinse with deionized water.

**Technical Note:**
This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

**Protocol Recommendations (ONCORE™ Automated Slide Staining System):**

- **OAI062 is intended for use with the ONCORE.** Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:
- **Protocol Name:** CK20
- **Protocol Template (Description):** Ms HRP Template 1
- **Dewaxing (DS Option):** DS2
- **Antigen Retrieval (AR Option):** AR2, low pH; 101°C
- **Reagent Name, Time, Temp.:** CK20, 30 min., 25°C

**Protocol Recommendations (Ventana Benchmark ULTRA):**

- **PM062 is compatible for use with the Benchmark ULTRA.** Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:
- **Template/Detection:** OptiView DAB IHC
- **Pretreatment Protocol:** CC1 64 minutes
- **Peroxidase:** Pre Primary Peroxidase Inhibitor
- **Primary Antibody:** 32 minutes, 36°C

**Limitations:**
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation
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Limitations Cont’d:
times and titers listed are not applicable to other detection systems, as results
may vary. The data sheet recommendations and protocols are based on
exclusive use of Biocare products. Ultimately, it is the responsibility of the
investigator to determine optimal conditions.

Quality Control:
Refer to CLSI Quality Standards for Design and Implementation of
Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-

Precautions:
1. This antibody contains less than 0.1% sodium azide. Concentrations less
than 0.1% are not reportable hazardous materials according to U.S. 29 CFR
1910.1200, OSHA Hazard communication and EC Directive 91/155/EC.
Sodium azide (NaN₃) used as a preservative is toxic if ingested. Sodium azide
may react with lead and copper plumbing to form highly explosive metal
azides. Upon disposal, flush with large volumes of water to prevent azide
build-up in plumbing. (Center for Disease Control, 1976, National Institute of
Occupational Safety and Health, 1976) (3)
2. Specimens, before and after fixation, and all materials exposed to them
should be handled as if capable of transmitting infection and disposed of with
proper precautions. Never pipette reagents by mouth and avoid contacting
the skin and mucous membranes with reagents and specimens. If reagents
or specimens come in contact with sensitive areas, wash with copious
amounts of water. (4)
3. Microbial contamination of reagents may result in an increase in
nonspecific staining.
4. Incubation times or temperatures other than those specified may give
erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the
vial.
6. The SDS is available upon request and is located at http://biocare.net.

Troubleshooting:
Follow the antibody specific protocol recommendations according to data
sheet provided. If atypical results occur, contact Biocare’s Technical Support
at 1-800-542-2002.

References:
1. Perry A, Parisi JE, Kurtin PJ. Metastatic adenocarcinoma to the brain: an
2. Sack MJ, Roberts SA. Cytokeratins 20 and 7 in the differential diagnosis of
Feb;16(2):132-6.
22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains
to Remove Azide Salts."
4. Clinical and Laboratory Standards Institute (CLSI). Protection of
Laboratory Workers from Occupationally Acquired Infections; Approved

VP Echelon Series antibodies are developed solely by Biocare Medical LLC
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