

**CDX2 + CK7****Prediluted Multiplex Cocktail (4-Step)**

Control Number: 902-367DS-090817

**Catalog Number:** APR 367 DS AA, H, L**Description:** 6.0, 25, 100 ml, prediluted**Dilution:** Ready-to-use**Diluent:** N/A**Intended Use:**

For Research Use Only. Not for use in diagnostic procedures.

**Summary and Explanation:**

CDX2 is a homeobox gene that encodes an intestine-specific transcription factor. Studies have shown it is expressed in the nuclei of epithelial cells throughout the intestine, from duodenum to rectum (3). The CDX2 protein is expressed in primary and metastatic colorectal carcinomas and has also been demonstrated in the intestinal metaplasia of the stomach and intestinal-type gastric cancer, while it is not expressed in the normal gastric mucosa (1). Studies have shown that CDX2 is a superior marker compared to CK20 and can be substituted in a panel of antibodies (2).

Cytokeratin 7 is a basic cytokeratin and is expressed in epithelial cells of ovary, lung and breast, but not in the colon or gastrointestinal tract (2). It is often used in conjunction with Cytokeratin 20 in distinguishing pulmonary ovarian and breast carcinomas (CK7+) from colon carcinomas (CK7-).

This Multiplex cocktail of CDX2 and CK7 can be used to distinguish colon cancers from breast, lung and ovarian cancers. CDX-2 will stain the nuclei brown and CK7 will stain target antigens red.

**Principle of Multiplex Staining:**

A Multiplex IHC stain can be accomplished in four major steps. The initial step consists of an antibody cocktail with at least one mouse and one rabbit antibody. This cocktail is applied to the tissue and will bind with two or more target antigens. A multiplex detection cocktail of horseradish peroxidase (HRP) and alkaline phosphatase (AP) conjugated secondary antibodies is applied. The third step consists of the addition of DAB-Substrate that binds to the HRP and produces a brown chromogenic reaction product. The fourth step consists of a Fast Red-Substrate that binds to the AP and produces a red chromogenic reaction product.

**Source:** Mouse Monoclonal and Rabbit Monoclonal**Species Reactivity:** Human; others not tested**Clone:** CDX2-88 + BC1**Isotype:** IgG1 and Rabbit IgG**Epitope/Antigen:** CDX2 and CK7**Cellular Localization:**

CDX2: (nuclear); brown

CK7: (cytoplasmic); red

**Positive Control:** Colon, breast, ovary and lung cancers**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. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

**Staining Protocol Recommendations:****Peroxide Block:**

Block for 5 minutes with Biocare's Peroxidized 1.

**Staining Protocol Recommendations Cont'd:****Pretreatment Solution (recommended):** Diva**Pretreatment Protocol:**

Heat Retrieval Method:

Retrieve sections under pressure using Biocare's Decloaking Chamber, followed by a wash in distilled water. Alternatively, steam tissue sections for 45-60 minutes. Allow solution to cool for 20 minutes then wash in distilled water.

**Protein Block (Optional):** Incubate for 10-15 minutes at RT with Biocare's Background Sniper.**Primary Antibody:**

Incubate for 30 minutes at RT.

**Double Stain Detection:**

Incubate for 30 minutes at RT using Biocare's MACH 2 Double Stain 2.

**Chromogen (1):** Incubate for 5 minutes at RT when using Biocare's Betazoid DAB.**Chromogen (2):**

Incubate for 10-20 minutes at RT with Biocare's Vulcan Fast Red. Rinse in deionized water.

**Counterstain:**

Rinse with deionized water. Incubate for 30-60 seconds with CAT Hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute.

**Technical Note:**

This antibody has been standardized with Biocare's MACH 2 Double Stain 2. It can also be used on an automated staining system. Use TBS buffer for washing steps.

**Limitations:**

This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

**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 (NaN<sub>3</sub>) 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) (4)
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. (5)
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 MSDS is available upon request and is located at <http://biocare.net/support/msds/>.

**Technical Support:**

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

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**References:**

1. Kim MJ. The usefulness of CDX-2 for differentiating primary and metastatic ovarian carcinoma: an immunohistochemical study using a tissue microarray. *J Korean Med Sci.* 2005 Aug;20(4):643-8.
2. Kennedy MT, *et al.* Expression pattern of CK7, CK20, CDX-2, and villin in intestinal-type sinonasal adenocarcinoma. *J Clin Pathol.* 2004 Sep;57(9):932-7.
3. Werling RW, Yaziji H, Bacchi CE, Gown AM. CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. *Am J Surg Pathol.* 2003 Mar;27(3):303-10.
4. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
5. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory workers from occupationally Acquired Infections; Approved guideline-Third Edition CLSI document M29-A3 Wayne, PA (2005).