



**Revision: 1** 

Rev. Date: Feb. 10, 2015

Page 1 of 3

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# CDX-2 & CEA Multiplex Cocktail; Clones EP25 & COL-1 (Ready-To-Use)

Availability/Contents:

<u>Item #</u> A00150-0002 A00150-0007 A00150-0025 <u>Volume</u> 2 ml 7 ml 25 ml

#### **Description:**

Species:	Rabbit and Mouse
Designation:	Cocktail of Rabbit and Mouse Monoclonal Antibodies to CDX-2 and CEA.
Immunogen:	Rabbits were injected with a synthetic peptide corresponding to residues near the C-terminus of human CDX-2. BALB/C mice were injected with Human colon carcinoma cell extracts (CEA).
Mol. Weight:	Unknown
Clone:	EP25 & COL-1
Isotype:	Rabbit IgG and Mouse IgG1, respectively.
Format:	This antibody has been pretitered and quality controlled to work on formalin-fixed paraffin- embedded as well as acetone fixed cryostat tissue sections. No further titration is required.
Specificity:	CDX-2 expression is restricted to nuclear staining in positive cells while CEA expression is restricted mostly to the lumen of colorectal crypts. The CEA antibody (COL-1) shows no reaction with a variety of normal tissues. CEA is not found in benign glands, stroma, or malignant prostatic cells. CEA positivity is seen in adenocarcinomas from the lung, colon, stomach, esophagus, gallbladder, urachus, salivary gland, ovary, and endocervix.
Background:	The caudal-related homeodomain protein 2 (CDX-2) which encodes an intestine-specific transcription factor is expressed in the nuclei of epithelial cells throughout the intestine, from duodenum to rectum.
	CDX-2 is thought to play an important role in the proliferation and differentiation of intestinal epithelial cells. The CDX-2 protein is expressed in primary and metastatic colorectal carcinomas, intestinal metaplasia of the stomach, and intestinal type gastric cancer. In human colorectal cancer, the expression of both CDX-2 and carbonic anhydrase 1, a gene regulated by CDX-2, is reduced or absent. However, CDX-2 is one of the important regulators in defining pathways seen in selected non-GI adenocarcinomas such as mucionous ovarian carcinomas and adenocarcinomas of the urinary bladder. CDX-2 is also used in diagnostic surgical pathology as a marker for gastrointestinal differentiation.
	The carcinoembryonic antigen (CEA) is a cell surface glycoprotein, normally expressed in fetal tissue but demonstrating only marginally in adults. It is a member of the immunoglobulin gene superfamily found on chromosome 19. The function and biological role of CEA is not clearly understood but evidently serves as an intracellular adhesion molecule. CEA is synthesized during development in the fetal gut and re-expressed in increased amounts in intestinal carcinomas and several other tumors. It is released from the apical surface of polarized columnar epithelial cells into the gut, whereas in deregulated tumor tissue, exfoliated CEA is useful in detecting early foci of gastric carcinoma and in distinguishing pulomonary adenocarcinomas (60-70% are CEA+) from pleural mesotheliomas (rarely or weakly CEA+).
Species Reactivity:	Human
<b>1</b>	•

Storage: 2° C



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### CE

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### Instructions For Use A00150-IFU-RUO

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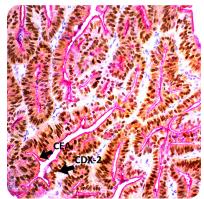
Page 2 of 3

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Positive Control:CDX-2: Colon for normal tissue and colon adenocarcinoma for abnormal tissue.<br/>CEA: Adenocarcinoma of colon, intestine, ovary, lung, cervix, gallbladder, stomach.Cellular Localization:CDX-2: Nuclear. CEA: Cell surface / cytoplasm.Titer/Working Dilution:No further dilution is required.Microbiological State:This product is not sterile.

#### **Uses/Limitations:**

Not to be taken internally. For research use only. This product is intended for qualitative immunohistochemistry with normal and neoplastic formalin-fixed, paraffin-embedded tissue sections, to be viewed by light microscopy. Do not use if reagent becomes cloudy. Do not use past expiration date. Non-Sterile.



Human colorectal carcinoma stained with Ultra-Tek HRP using DAB Chromogen and UltraTek Alk-Phos using Permanent Red Chromogen.

#### **Procedure:**

1. Deparaffinize and rehydrate tissue section.

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- 2. **Tissue Section Pretreatment (Highly Recommended):** Staining of formalin fixed, paraffin embedded tissue sections is enhanced by pretreatment with Citrate Plus (ScyTek catalog# CPL500).
- 3. Wash 2 times in DI/Distilled water.
- 4. To reduce nonspecific background staining due to endogenous peroxidase, incubate slide in hydrogen peroxide for 10-15 minutes.
- 5. Wash 2 times in buffer.
- 6. Apply Super Block (Catalog# AAA) and incubate for 5 minutes at room temperature to block nonspecific background staining. **Note:** Do not exceed 10 minutes or there may be a reduction in desired stain.
- 7. Wash 3 times in buffer.
- 8. Apply primary antibody cocktail and incubate for 30 minutes in a humid environment.
- 9. Wash 3 times in buffer.
- 10. Apply UltraTek Anti-Mouse (Catalog# ABJ) and incubate for 10 minutes at room temperature.
- 11. Wash 3 times in buffer.
- 12. Apply UltraTek HRP (Catalog# ABL) and incubate for 10 minutes at room temperature.
- 13. Rinse 3 times in buffer.
- 14. Rinse 1 time in DI/Distilled water.
- 15. Apply mixed DAB Chromogen / DAB Substrate (Catalog# ACV) and incubate for 10-15 minutes, depending on the desired stain intensity.

WARNING: DAB is a suspected carcinogen. Handle with care and dispose of according to all regulations.

- 16. Rinse 3 times in buffer.
- 17. Apply UltraTek Anti-Rabbit (Catalog# ABK) and incubate for 10 minutes at room temperature.
- 18. Rinse 3 times in buffer.

Storage: 2° C



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**Revision: 1** 

Rev. Date: Feb. 10, 2015

Page 3 of 3

P.O. Box 3286 - Logan, Utah 84323, U.S.A. - Tel. (800) 729-8350 - Tel. (435) 755-9848 - Fax (435) 755-0015 - www.scytek.com

- 19. Rinse 1 time in DI/Distilled water.
- 20. Apply UltraTek Alk-Phos (Catalog# ABM) and incubate for 15 minutes at room temperature.
- 21. Rinse 3 times in buffer.
- 22. Rinse 1 time in DI/Distilled water.
- 23. Apply mixed Permanent Red Concentrate / Permanent Red Substrate (Catalog# PRD) and incubate for 10-15 minutes, depending on the desired stain intensity.
- 24. Rinse 2 times in DI/Distilled water.
- 25. Counterstain with Hematoxylin (Catalog# HMM) and coverslip using a permanent mounting media.

**Precautions:** Contains Sodium Azide as a preservative (0.09% w/v).

Do not pipette by mouth.

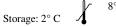
Avoid contact of reagents and specimens with skin and mucous membranes. Avoid microbial contamination of reagents or increased nonspecific staining may occur. This product contains no hazardous material at a <u>reportable concentration</u> according to U.S. 29 CFR 1910.1200, OSHA Hazardous Communication Standard and EC Directive 91/155/EC.

#### **References:**

- 1. Drummond F, et al. Ann Hum Genet 61(5): 393-400.
- 2. Gregory PA, et al. Pharmacogenet Genomics 16(7): 527-36, 2006.
- 3. Werling RW, Yaziji H, Bacchi CE, Gown AM. Am J Surg Pathol. 27(3): 303-10, 2003.
- 4. Cerna M, Holubec Jr L, Pesta M et al. Anti-Cancer Res. 26: 803-808, 2006.

Note: CDX-2 bearing EP Clone EP25 is Manufactured using Epitomics's RabMAb® technology under U.S. Patent Nos. 5,675,063 and 7,402,409.

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