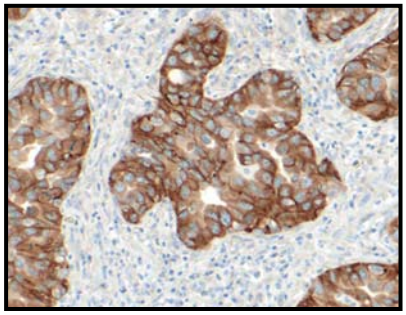
CONFIRM® anti-EGFR (5B7) Rabbit Monoclonal Primary Antibody

Catalog number 790-4347



INTENDED USE

This antibody is intended for *in vitro* diagnostic (IVD) use.

Ventana Medical Systems' (Ventana) CONFIRM anti-EGFR (5B7) Rabbit Monoclonal Antibody is a primary antibody designed to qualitatively detect the presence of the internal domain of EGFR via light microscopy in formalin fixed, paraffin embedded tissue following staining on a Ventana automated slide stainer. This antibody is directed against the internal domain of the human Epidermal Growth Factor Receptor (also called EGFR, ErbB1 or HER 1). The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests.

SUMMARY AND EXPLANATION

CONFIRM anti-EGFR (5B7) is a rabbit monoclonal antibody produced against a synthetic peptide from the carboxy terminal (cytoplasmic) region of EGFR. The epitope recognized by anti-EGFR (5B7) is associated with an internal domain of EGFR, and therefore EGFR staining with antibodies that recognize extracellular domains may not always correlate with CONFIRM anti-EGFR (5B7) staining.

EGFR is a member of the receptor tyrosine kinase HER family. Also known as HER 1, EGFR is structurally related to HER 2, HER 3, and HER 4. Members of this protein family are capable of forming heterodimers to mediate signal transduction. EGFR is expressed at low levels in most normal epithelial tissues, but can be overexpressed in cancers of the breast, brain, bladder, lung, gastric, head and neck, esophagus, cervix, ovary, endometrium, and particularly in squamous cell carcinomas.[1]

In Western blots of A431 cells, rabbit monoclonal anti-EGFR (5B7) recognizes the Mr 170 kD wild type EGFR. Specificity to EGFR was confirmed by peptide inhibition studies where antibody was incubated with a synthetic EGFR peptide. The peptide inhibited antibody binding to EGFR in tissue. The cellular staining pattern for EGFR is membrane and cytoplasmic.

REAGENT PROVIDED

CONFIRM anti-EGFR (5B7) contains sufficient reagent for staining 50 slides.

1 – 5 mL dispenser of CONFIRM anti-EGFR (5B7) contains approximately 2 µg of a rabbit monoclonal antibody.

The antibody is diluted in 0.05 M Tris-HCl with 2% carrier protein, and 0.10% ProClin® 300, a preservative containing the active ingredients 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one.

Total protein concentration of the reagent is approximately 10.5 µg/ mL. Specific antibody concentration is approximately 0.4 µg/ mL. There is no known irrelevant antibody reactivity observed in this product.

There is a trace (~2%) of fetal calf serum of U.S. origin from the stock solution.

Refer to the appropriate Ventana detection kit package insert for detailed descriptions of: (1) Principles and Procedures, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Preparation, (4) Quality Control, (5) Troubleshooting, (6) Interpretation of Staining, and (7) General Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents such as Ventana detection kits (for example: *ultra*View™ Universal DAB) rabbit negative control reagent, and ancillary components, including negative and positive tissue control slides, are not provided.

STORAGE

Store at 2-8° C. Do not freeze.

To ensure proper reagent delivery and stability of the antibody, after every run the cap must be replaced and the dispenser must be immediately placed in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed, formalin fixed, paraffin embedded tissues are suitable for use with this primary antibody when used with Ventana detection kits and a Ventana automated slide stainer. The recommended tissue fixative is 10% neutral buffered formalin.[2] Heat induced epitope retrieval with a basic pH (~8.0) buffer is recommended. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

There are no definitive signs to indicate instability of this product; therefore, positive and negative controls should be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
3. Avoid microbial contamination of reagents.
4. Consult local or state authorities with regard to recommended method of disposal.
5. The preservative in the reagent is ProClin 300. Symptoms of overexposure to ProClin 300 include skin and eye irritation, and irritation of mucous membranes and upper respiratory tract. The concentration of ProClin 300 in this product is less than or equal to 0.10% and does not meet the OSHA criteria for a hazardous substance. Systemic allergic reactions are possible in sensitive individuals.

STAINING PROCEDURE

Ventana primary antibodies have been developed for use on a Ventana automated slide stainer in combination with Ventana detection kits and accessories. A recommended staining protocol for the BenchMark® series with *ultra*View Universal DAB Detection Kit (Cat. No. 760-500) is listed below in Table 1. The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument's Operator's Manual. Refer to *ultra*View Universal DAB Detection Kit (Cat. No. 760-500) package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for CONFIRM anti-EGFR (5B7) with *ultra*View Universal DAB Detection Kit on BenchMark Series

Procedure Type	Method
	BenchMark Series
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard
Enzyme (Protease)	None required
Antibody (Primary)	Approximately 16 Minutes, 37° C
Ultra Wash	Optional
Counterstain	Hematoxylin II, 2 to 4 Minutes
Post Counterstain	Bluing, 2 to 4 Minutes

Due to variation in tissue fixation and processing, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens and detection used. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".[3]

POSITIVE TISSUE CONTROL

Examples of positive control tissues for CONFIRM anti-EGFR (5B7) are skin, non small cell lung carcinoma (as depicted in image above) and placenta. Positive membrane and cytoplasmic staining of basal epithelial cells, exocrine sweat glands, sebaceous glands, dermal fibroblasts, and perineurium can be present in skin. Synciotrophoblasts of the placenta stain positively.

STAINING INTERPRETATION

The cellular staining pattern for EGFR is membrane and cytoplasmic.

SPECIFIC LIMITATIONS

This antibody has been optimized for a 16 minute incubation time on BenchMark XT in combination with *ultra*View Universal DAB Detection Kit (Cat. No. 760-500), however the user must validate use of this reagent.

PERFORMANCE CHARACTERISTICS

1. Specificity of CONFIRM anti-EGFR (5B7) was determined by testing formalin fixed, paraffin embedded normal and neoplastic tissues. For normal tissues, results are as follows: Adrenal gland (0/1), bone marrow (0/3), brain cerebrum (0/3), brain cerebellum (3/3), breast (3/3), cervix (0/3), colon (3/3), esophagus (3/3), heart (2/3), intestine (3/3), kidney (0/3), liver (0/3), lung (3/3), lymph node (0/3), mesothelium (2/3), nerve (0/3), ovary (2/3), pancreas (0/2), parathyroid (0/3), pituitary (1/3), prostate (0/2), salivary gland (0/3), skin (3/3), spleen (1/3), stomach (0/3), striated muscle (0/2), testis (0/2), thymus (3/3), thyroid (0/3), tonsil (3/3), and uterus (0/3).For neoplastic tissues, results are as follows: Atypical meningioma (1/1), glioblastoma (1/1), ependymoma (0/1), oligodendroglioma (1/1), ovarian serous papillary adenocarcinoma (0/1), ovarian mucous papillary adenocarcinoma (1/1), islet cell carcinoma (0/1), pancreatic adenocarcinoma (1/1), testicular seminoma and embryonal carcinoma (0/2), medullary thyroid carcinoma (0/1), papillary thyroid carcinoma (1/1), intraductal, lobular, and infiltrating breast carcinoma (0/3), diffuse B-cell lymphoma in spleen (0/1), small cell lung carcinoma (0/1), squamous cell lung carcinoma (0/1), lung adenocarcinoma (1/1), esophageal squamous cell and adenocarcinoma (2/2), adenocarcinoma in stomach (0/1), intestinal adenocarcinoma and mesenchymoma (2/2),colorectal adenocarcinoma and mesenchymoma (2/4), hepatocellular carcinoma (1/1), hepatoblastoma (0/1), adenocarcinoma in prostate (0/1), transitional cell carcinoma in prostate (1/1), uterine leiomyoma (0/1), endometrial carcinoma (1/1), uterine clear cell and squamous carcinomas (0/3), embryonal rhabdomyosarcoma (0/1), rectal melanoma (0/1), basal cell carcinoma (0/1), Hodgkin's lymphoma (0/1), and diffuse type lymphoma (0/2).
2. For evaluation of sensitivity, a cohort of non-small cell lung carcinomas (NSCLC) was stained on a BenchMark XT with CONFIRM anti-EGFR (5B7) and 32/47 cases (68%) stained positively for EGFR. This is consistent with the published expression of EGFR in NSCLC.[4]
3. Inter lot reproducibility was determined by testing three lots of CONFIRM anti-EGFR (5B7) on four tissue samples representing the dynamic range of EGFR expression on a BenchMark XT. Three out of three lots tested scored equivalently across all four tissue samples.
4. CONFIRM anti-EGFR (5B7) is compatible with NexES®, BenchMark, and BenchMark XT instrument systems and *i*VIEW™ DAB, Enhanced Alkaline Phosphatase Red, *ultra*View Universal DAB, and *ultra*View Universal Red detection chemistries.

REFERENCES

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3. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. (NR Rose Ed.) ASM Press, 2002.
4. Selvaggi G, Novello S, Torri V, Leonardo E, De Giuli P, Borasio P, Mossetti C, Ardissone F, Lausi P, Scagliotti GV. Epidermal growth factor receptor overexpression correlates with a poor prognosis in completely resected non-small-cell lung cancer. Ann Oncol. 15(1):28-32, 2004.

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