Rabbit anti-phospho-EGFr (Tyr1173)

For Research Use Only
Lot No.

[X] 18-2465 0.2 mL Concentrate Antibody

INTENDED USE

For research use only. Not for use in diagnostic procedures.

Invitrogen's polyclonal Rabbit anti-phospho-EGFr (Tyr1173) antibody is intended to qualitatively stain EGFr when it is phosphorylated at tyrosine residue 1173 in frozen and formalin-fixed, paraffin-embedded tissue sections.

SPECIFICITY AND REACTIVITY

Epidermal growth factor receptor (EGFr) is a single-pass transmembrance tyrosine kinase that is involved in the regulation of growth in many animal cells, including cancer cells. Ligand binding to this receptor results in receptor dimerization, autophosphorylation, activation of various downstream signaling molecules and lysomsomal degradation.[1] Over-expression of EGFr has been observed in many types of neoplasia, as the result of gene amplification and/or increased protein transcription. EGFr over-expression has been detected in endometrial carcinoma, correlated with myometrial invasion,[2] in lung neoplasms[3,4] glioblastoma multiforme brain tumors[5], and in most head and neck carcinomas[6].

The levels of EGFr phosphorylation are among the indicative factors used for the selection of patients for EGFr-targeted therapy.[7] Identified autophosphorylation sites of EGFr are tyrosine residues at 992, 1068, 1086, 1148, and 1173 of the C-terminal tail of the receptor molecule.[8]

REAGENT PROVIDED

Rabbit anti-phospho-EGFr (Tyr1173) is purified from rabbit antisera and diluted in phosphate buffered saline (PBS), pH 7.4, and 1% bovine serum albumin (BSA) with 0.1% sodium azide (NaN_3) as a preservative.

Immunogen: phospho-EGFr (Tyr1173)-peptide-KLH conjugate

Total protein concentration: g/L

PAD: ZMD.506

Antibody concentration: mg/L

STORAGE: 2-8°C

POSITIVE CONTROL TISSUE: Esophageal carcinoma or glioblastoma

EXPECTED STAINING PATTERN: Membrane with some cytoplasm

INSTRUCTIONS FOR USE

PRETREATMENT REQUIREMENTS:

Epitope Retrieval: Required (EDTA pH 8.0) (See page 2 for protocol)
Enzyme Digestion: Not required

Rabbit anti-phospho-EGFr (Tyr1173) may be diluted according to Table 1 when using the Invitrogen detection systems below.

Table 1. Dilution Table

Invitrogen Kit	Predilute Antibody	Dilution for Concentrate	Incubation Time
Histostain-SP or SAP kits*	Ready-To-Use	1: 50 - 1: 100	60 min.
Histostain®-*Plus* Kits	Ready-To-Use	1: 100 - 1: 200	30-60 min.
SuperPicTure™ Polymer Kits	Ready-To-Use	1: 100 - 1: 200	30-60 min.
Cap-*Plus*™ Kits	Ready-To-Use	1: 100 - 1: 200	30-60 min.

*** Use Histostain-SP or -SAP kits only for Cat. No. 08-0XXX and 18-X001 to 18-X200 primary antibodies.**

This is a guideline only. Optimal antibody concentrations may vary based on specimen and preparation method used, and should be determined by each individual laboratory.

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SPECIMEN PREPARATION

1. Use tissue fixed in 10% neutral buffered formalin or other fixative on regular basis, or frozen tissue sections.
2. Cut 3-4 µm sections and place on positively charged slides.
3. Dry overnight at 37° C or for 2-4 hrs at 58°C.

PRETREATMENT

Heat Induced Epitope Retrieval (HIER), if required

1. Deparaffinize slides. Tissue sections should be mounted on silane, poly-L-Lysine, or HistoGrip (Cat. No. 00-8050) coated slides.
2. Wash slides with distilled water 3 times for 2 min each.
3. Place a 1L glass (Pyrex) beaker containing 500 ml of 0.01 M citrate buffer (Cat. No. 00-5000) or EDTA solution (Cat. No. 00-5500) on a hot plate. Heat the buffer solution until it boils. *(This step may be prepared before slide deparaffinization, as the buffer may take several minutes to boil).*
4. Put the slides in a slide rack and place in the beaker with boiling buffer. Keep it boiling for 15 minutes.
5. After heating, remove beaker from the hot plate and allow it to cool down for at least 15-20 minutes at room temperature.
6. Rinse slides with PBS (Cat. No. 00-3000) and begin the immunostaining protocol.

Enzyme Digestion, if required

1. Prewarm the enzyme of choice at 37ºC for 10 min.
2. Add the prewarmed enzyme to a tissue section and incubate at 37ºC for 10 min.
3. Wash in several changes of PBS (Cat. No. 00-3000) and begin the immunostaining protocol.

RECOMMENDED MANUAL STAINING PROCEDURE

1. Submerge slides in peroxidase quenching solution and rinse with PBS.
2. Apply serum blocking solution.
3. Apply primary antibody and incubate for 30-60 min at room temperature; rinse with PBS.
4. Apply secondary antibody and incubate for 10 min at room temperature; rinse with PBS.
5. Apply enzyme conjugate and incubate for 10 min at room temperature; rinse with PBS.
6. Apply chromogen and incubate for 5-10 min at room temperature; rinse with PBS.

MATERIALS REQUIRED BUT NOT PROVIDED

	Reagent	Catalog No.
1.	HistoGrip™	00-8050
2.	Super PAP Pen	00-8899
3.	Isotype Control for Rabbit or Mouse Primary Antibody	08-6199 or 08-6599
4.	Antibody Diluent	00-3118
5.	PBS (0.01 M PBS)	00-3000
6.	Mayer's Hematoxylin	00-8011
7.	Citrate Buffer pH 6.0 (**if required for HIER**)	00-5000
8.	EDTA Solution (**if required for HIER**)	00-5500
9.	Digest-All™ 1, Digest-All™ 2, or Digest-All™ 3 (**if required for Enzyme Digestion**)	00-3007 or 00-3008 or 00-3009

10. *SuperPicTure*™ polymer kit, or LAB-SA kit (Histostain®-*Plus*, and Cap-*Plus*™).
11. Chromogen/substrate (if not included in detection kit): *Single Solution* AEC (Cat. No. 00-1111), or DAB (Cat. No. 00-2014), or Fast-Red (Cat. No. 00-2234).
12. Mounting solution: Histomount™ (for DAB: Cat. No.00-8030), GVA (for AEC, or Fast-Red: Cat. No. 00-8000), or Clearmount™ (for AEC, DAB, or Fast-Red: Cat. No. 00-8010).

REFERENCES

1. Hackel PO, et al. *Curr Opin Cell Biol* 11(2):184-189, 1999.
2. Niikura H, et al. *Human Pathol* 27(3):282-289, 1996.
3. Ozane B, Richardson CS. *J Pathol* 149:191-201, 1986.
4. Rusch V, et al. *Ann Thorac Surg* 62:798-810, 1996.
5. Agosti RM, et al. *Virchows Arch Pathol Anat* 420:321-325, 1992.
6. Christensen ME. *Danish Med Bull* 45(2):121-134, 1998.
7. Kanematsu T, et al. *Oncol Res* 13(5):289-298, 2003.
8. Downward J, et al. *Nature* 311(5985):483-485, 1984.

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