Rabbit anti-phospho-Akt (Ser473)

For Research Use Only
Lot No.

[X] 18-2484 0.2 mL Concentrate Antibody

INTENDED USE

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

Invitrogen's monoclonal Rabbit anti-phospho-Akt (Ser473) antibody is intended to qualitatively stain Akt when it is phosphorylated at serine residue 473 in frozen and formalin-fixed, paraffin-embedded tissue sections.

SPECIFICITY AND REACTIVITY

Akt, also known as Protein Kinase B (PKB), is a 65 kDa serine/threonine kinase that plays an important role in diverse biological responses such as regulation of metabolism, cell survival and growth. Phosphorylation at serine 473 is required for full activation of Akt.[1] Recent studies have shown that positive phospho-Akt (ser473) is a favorable prognostic factor in non-small cell lung carcinoma, and may help select patients who will benefit from EGFr-targeted therapy such as gefitinib.[2-3]

REAGENT PROVIDED

Rabbit anti-phospho-Akt (Ser473) is purified and diluted in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol and 1 mg/ml bovine serum albumin (BSA, IgG, protease free) with 0.1% sodium azide (NaN_3) as a preservative.

Immunogen: phospho-Akt (Ser473)-peptide

Clone: 14-6

Total protein concentration: g/L

Antibody concentration: mg/L

STORAGE: 2-8°C

POSITIVE CONTROL TISSUE: Breast cancer

EXPECTED STAINING PATTERN: Nuclear, cytoplasmic

INSTRUCTIONS FOR USE

PRETREATMENT REQUIREMENTS:

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

Rabbit anti-phospho-Akt (Ser473) may be diluted according to Table 1 when using the Invitrogen detection systems below.

RECOMMENDED INCUBATION TIME: Overnight at 4°C

Table 1. Dilution Table

Invitrogen Kit	Predilute Antibody	Dilution for Concentrate	Incubation Time
Histostain®-*Plus* Kits	Ready-To-Use	1: 25 - 1: 50	Overnight at 4°C
SuperPicTure™ Polymer Kits	Ready-To-Use	1: 25 - 1: 50	Overnight at 4°C
Cap-*Plus*™ Kits	Ready-To-Use	1: 25 - 1: 50	Overnight at 4°C

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.

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 overnight at 4°C; 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. Meier R, et al. *J Biol Chem* 272(48):30491-7, 1997.
2. Shah A, et al. *Clin Cancer Res* 11(8):2930-6, 2005.
3. Cappuzzo F, et al. *J Natl Cancer Inst* 96(15):1133-41, 2004.

TRADEMARKS