Rabbit (polyclonal) Anti-Akt/PKB [pS\textsuperscript{473}]
Phosphorylation Site Specific Antibody
Alexa Fluor\textsuperscript{®} 488 Conjugate

PRODUCT ANALYSIS SHEET

<table>
<thead>
<tr>
<th>Catalog Number:</th>
<th>44-622A1 (50 Tests)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot Number:</td>
<td>See product label</td>
</tr>
<tr>
<td>Volume:</td>
<td>100 μL</td>
</tr>
<tr>
<td>Suggested Dilutions:</td>
<td>The optimal dilution should be determined empirically for each cell type and stimulation protocol. The recommended starting dilution for most experimental systems is 1:50.</td>
</tr>
<tr>
<td>Formulation:</td>
<td>Alexa Fluor\textsuperscript{®} 488-conjugated purified immunoglobulin in Dulbecco’s phosphate buffered saline (without Mg\textsuperscript{2+} and Ca\textsuperscript{2+}), pH 7.3 (+/- 0.1), with 0.2% BSA and 0.05% sodium azide. (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)</td>
</tr>
<tr>
<td>Target Summary:</td>
<td>Akt, also known as Protein Kinase B (PKB) or RAC-\textalpha, 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 by phosphorylating many proteins including GSK-3\textbeta, caspase 9, BAD, and the Forkhead Transcription Factor. Akt is activated by PI3K, which in turn can interact with proteins such as FAK (when FAK is phosphorylated at tyrosine 397) thereby linking activation to the cytoskeleton. Akt is phosphorylated on threonine 308 by PDK1 and on serine 473 by PDK2. Phosphorylation at serine 473 is required for full activation of Akt.</td>
</tr>
<tr>
<td>Reactivity:</td>
<td>Human and mouse Akt.</td>
</tr>
</tbody>
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**Immunofluorescence Staining:**
NIH3T3 cells were left untreated (A), stimulated with PDGF (50 ng/mL for 15 minutes) (B), or pretreated with the PI3-K inhibitor, wortmannin, followed by PDGF stimulation (C). Cells were fixed prior to immunostaining with this anti-Akt [pS\textsuperscript{473}] Alexa Fluor\textsuperscript{®} 488 conjugate. The data show that Akt is activated in response to PDGF stimulation, and that this activation is blocked in cells pre-treated with wortmannin.

Please visit our website (Invitrogen.com) to view the images in full color.

A. NIH3T3 (Untreated)  B. NIH3T3+PDGF

C. NIH3T3 + Wortmannin + PDGF

This product is for research use only. Not for use in diagnostic procedures.

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Production: The antibody was produced against a chemically synthesized phosphopeptide derived from the region of Akt that contains serine 473. The antibody was affinity purified by sequential epitope-specific chromatography, then conjugated to Alexa Fluor® 488 under optimal conditions.

Alexa Fluor® 488 : Protein Ratio: 2.43 : 1

Storage: Store at 2-8°C for up to one month. For long term storage, apportion the antibody into working aliquots and store at –20°C. Avoid repeated freeze-thaw cycles to prevent denaturing the antibody. Protect from light.

Expiration Date: Expires one year from date of receipt when stored as instructed.

Related Products: Antibodies:

- Anti-Akt/PKB, Cat. # 44-609G
- Akt/PKB [pS473], Cat. # 44-622G
- Akt/PKB [pT308], Cat. # 44-602G
- PRAS40 [pT246], Cat. # 44-1100G
- AS-160 [pT642], Cat. # 44-1071G
- Caspase-9 [315/316], Cat. # 44-692
- PTEN [pS370], Cat. # 44-1060G
- Alexa Pyk2 [pY402], Cat. # 44-618A1
- Alexa 488 JNK1/2 [pTpY183/185], Cat. # 44-682A1
- Alexa 488 ERK1/2 [pTpY185/187], Cat. # 44-680A1
- Alexa 488 PRAS40 [pT246], Cat. # 44-1100A1
- Alexa 488 PTEN [pSpTpS380/382/385], Cat. #44-1066A1
- Alexa 488 Src [pY418], Cat. # 44-660A1

References:


The Alexa Fluor® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc., for research use only, except for use in combination with microarrays.

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Immunofluorescence Staining of Adherent Cells:

Invitrogen’s phosphorylation site specific antibodies have been used successfully in many antibody-based techniques, including Western blot analyses and sandwich immunooassays (i.e., phosphoELISA™ and Lumitex™ assays). When used in immunohistochemistry, these antibodies not only detect phosphorylation events, but also provide valuable information about the subcellular localization of phosphorylated proteins. Invitrogen’s Alexa Fluor® 488-conjugated phosphorylation site specific antibodies have been developed specifically to provide a facile method for detecting protein phosphorylation and localization by immunohistochemistry.

Procedure:

1. Plate adherent cells onto glass cover slips.
2. Culture the cells for 16 hours in appropriate medium. It is important to note that serum starvation may be necessary in some stimulation procedures.
3. Stimulate the cells as desired.
4. Remove the medium from the cells by decanting.
5. Fix the cells by pipetting 200 μL 95% ice cold methanol onto the slides. (Fixatives composed of equal volumes of acetone and methanol, or 4% paraformaldehyde, have also been used successfully.)
6. Incubate for 10 minutes at -20°C.
7. Remove the fixative solution by decanting.
8. Pipette 200 μL Blocking Buffer onto the slides. (Blocking Buffer Formulation: 3% BSA/TBST/0.1% Triton X-100, supplemented with protease inhibitor cocktail [Sigma Cat. # P8340] and phosphatase inhibitor cocktail I and II [Sigma Cat. # P2850 and P5726]).
9. Incubate for 30 minutes at room temperature.
10. Remove the Blocking Buffer by decanting.
11. Pipette 200 μL Alexa Fluor® 488 conjugated phosphorylation site specific antibody, diluted in Blocking Buffer, onto the cover slip.
12. Incubate for two hours at room temperature or overnight at 2-8°C.
13. Remove the antibody solution by decanting.
14. Wash the cells twice with phosphate buffered saline, pH 7.2, 10 minutes each.
15. Add one drop of Vectashield solution (Vector Lab, H-1500) to prevent photobleaching the fluorescent signal. Vectashield solution contains DAPI to counterstain the nucleus. Mount the cover slip on a microscopic slide.
16. Examine the slides with an immunofluorescence microscope (e.g., Zeiss Axioplan 2). We suggest using the 100x oil immersion lens.