Rabbit (polyclonal) Anti-c-Fos [pT^{325}] Phosphospecific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number:	44-281G (10 mini-blot size)
Lot Number:	See product label
Volume:	100 µL
Form of Antibody:	Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier.
Preservative:	0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)
Purification:	Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated c-Fos. The final product is generated by affinity chromatography using a c-Fos-derived peptide that is phosphorylated at threonine 325.
Immunogen:	The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human c-Fos that contains threonine 325. The sequence is conserved in human, mouse and rat.
Target Summary:	c-Fos is a ~60 kDa immediate early gene product and a member of AP-1 family. c-Fos dimerizes with c-Jun to form the transcriptionally active complex AP-1, and functions in regulating cell proliferation and differentiation. c-Fos expression and activation are induced in many tumors (including breast cancer), and in response to a variety of stimuli (including growth factors, cytokines and stress). ERK1&2 mediate growth factor-induced c-Fos induction and transcripional activation by directly phosphorylating the carboxyl terminal domain of c-Fos. c-Fos is phosphorylated on several serine and threonine sites, including threonine 325 and 232. Phosphorylation of threonine 325 is believed to augment c-Fos transcriptional activity.
Reactivity:	Human and mouse c-Fos. Rat c-Fos (100% homologous) has not been tested, but is expected to react.
Applications:	The antibody has been used for Western blotting applications. Other applications have not been tested at Invitrogen.
Suggested Working Dilutions:	For Western blotting applications, we recommend using the antibody at a 1:1000 starting dilution. The optimal antibody concentration should be determined empirically for each specific application.
Storage:	Store at –20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at –20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.
Expiration Date:	Expires one year from date of receipt when stored as instructed.
Positive Control Used:	Human epidermoid carcinoma (A431) cells -/+ EGF or 15% serum; NMuMG cells -/+ TGFβ.

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Related Products:

Antibodies:

c-Fos [pT^{232}], Cat. # 44-280G	Anti-Akt/PKB pan, Cat. # 44-609G
Elk-1 [pS^{383}], Cat. # 44-238G	Akt/PKB [pT^{308}], Cat. # 44-602G
NFκB [pS^{529}], Cat. # 44-711G	Akt/PKB [pS^{473}], Cat. # 44-621G
c-Jun [pS^{73}], Cat. # 44-292G	ERK5 [pTpY$^{218/220}$], Cat. # 44-612G
SHP2 [pY^{542}], Cat. # 44-554G	ERK1&2 [pTpY$^{185/187}$], Cat. # 44-680G
SHP2 [pS^{576}], Cat. # 44-557G	NFAT-1 [pS^{54}], Cat. # 44-944G
	Anti-Grb2 pan, Cat. # AHO0512

References:

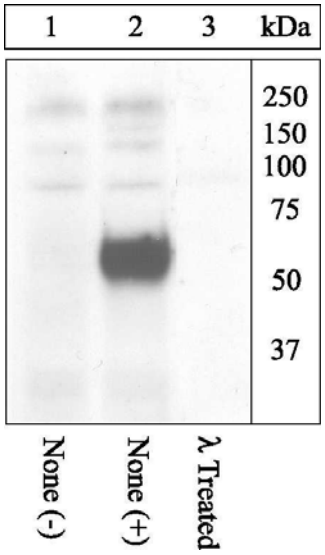
Lu, Y., et al. (2004) Overexpression of ErbB2 receptor inhibits IGF-I-induced Shc-MAPK signaling pathway in breast cancer cells. Biochem. Biophys. Res. Commun. 313(3):709-715.

Monje, P., et al. (2003) Phosphorylation of the carboxyl-terminal transactivation domain of c-Fos by extracellular signal-regulated kinase mediates the transcriptional activation of AP-1 and cellular transformation induced by platelet-derived growth factor. Mol. Cell. Biol. 23(19):7030-7043.

Mikula, M., et al. (2003) The proto-oncoprotein c-Fos negatively regulates hepatocellular tumorigenesis. Oncogene 22(43):6725-6738.

Wang, L.H., et al. (2003) The cis decoy against the estrogen response element suppresses breast cancer cells via target disrupting c-fos not mitogen-activated protein kinase activity. Cancer Res. 63(9):2046-2051.

Deng, T. and M. Karin (1994) c-Fos transcriptional activity stimulated by H-Ras-activated protein kinase distinct from JNK and ERK. Nature 371(6493):171-175.



Phosphatase Stripping

Extracts of A431 cells left untreated (lane 1) or treated with 2.0 µg/ml of EGF for one hour (lanes 2 & 3) were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. The membrane was left untreated (lanes 1 & 2) or treated with Lambda phosphatase (lane 3), blocked with a 3% Milk-TBST buffer for one hour at room temperature, and then incubated with the c-Fos [pT^{325}] antibody for two hours at room temperature in a 3% Milk-TBST buffer. After washing, the membrane was incubated with goat F(ab')$_2$ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and signals were detected using the Pierce SuperSignal™ method.

The data show that the phosphorylation of c-Fos on threonine 325 is induced by EGF treatment in this cell system, and that phosphatase treatment eliminates the signal, demonstrating the phospho-specificity of the antibody.

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Western Blotting Procedure

1. Lyse approximately 10^7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Cat. # FNN0011). Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH_2O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the PVDF membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) overnight at 4°C or for one hour at room temperature.
12. Incubate the blocked blot with primary antibody at a 1:1000 starting dilution in Tris buffered saline supplemented with 3% non-fat dried milk and 0.1% Tween 20 overnight at 4°C or for two hours at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase conjugate (Cat. # ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404) in conjunction with your chemiluminescence reagents and instrumentation.

Cell Lysis Buffer
Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM Na_3VO_4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO) or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

Transfer Buffer
Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add
1 mL 10% SDS.
Cool to 4°C prior to use.

Tris Buffered Saline
Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer
Formulation:
100 mL Tris buffered saline
3 gm non-fat dried milk
0.1 mL Tween 20

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