Rabbit (polyclonal) Anti-CK2β [pS^{209}] Phosphospecific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number:	44-1090G (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 CK2β. The final product is generated by affinity chromatography using a CK2β-derived peptide that is phosphorylated at serine 209.
Immunogen:	The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human CK2β that contains serine 209. The sequence is conserved in mouse, rat, chicken, pig and rabbit.
Target Summary:	Protein kinase CK2 or PKCK2 (formerly known as casein kinase 2) is a serine/threonine kinase, and consists of two constitutively active catalytic (α and α') and two identical regulatory (β) subunits that form an active tetrameric holozyme. CK2 is involved in an array of cellular responses including cell survival, neoplasia and viral infections, and mediates its survival and anti-apoptotic role in part by protecting cellular proteins from caspase-mediated degradation via their phosphorylation. CK2β (25 kDa) undergoes phosphorylation at serines 2 and 3 in the N-terminal region and serine 209 in the carboxyl terminus. CK2β serine 209 is phosphorylated by p34cdc2 during mitosis.
Reactivity:	Human CK2β. Other species CK2β (100% homologous) have not been tested, but are expected to react. This antibody does not cross react with CK2α [pS^{360}] (mouse), [pS^{362}], [pS^{370}], or CK2β [pS^{2}].
Applications:	The antibody has been used in Western blotting. 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:	HeLa cell lysates or K562 cell lysates.

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

www.invitrogen.com
Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com

This antibody is manufactured under a licensed process covered by Patent # 5, 599, 681.

PI441090G (Rev 11/08) DCC-08-1089

Related Products:

Antibodies:

PTEN [pS^{370}], Cat. # 44-1060G	HSP27 [pS^{82}], Cat. # 44-534G
PTEN [pSpTpS$^{380/382/385}$], Cat. # 44-1066G	ERK1&2 [pTpY$^{185/187}$], Cat. # 44-680G
BID [59/60] CSSA, Cat. # 44-436G	GSK-3β [pS^{9}], Cat. # 44-600G
BID [p15] CSSA, Cat. # 44-433G	Akt/PKB [pT^{308}], Cat. # 44-602G
β-Catenin [pS^{45}], Cat. # 44-208G	Akt/PKB [pS^{473}], Cat. # 44-623G
NFκB [pS^{529}], Cat. # 44-711G	Akt/PKB, Cat. # 44-609G

References:

Miyata, Y. and E. Nishida (2004) CK2 controls multiple protein kinases by phosphorylating a kinase-targeting molecular chaperone, Cdc37. Mol. Cell. Biol. 24(9):4065-4074.

Lee, G., et al. (2004) Casein kinase II-mediated phosphorylation regulates alpha-synuclein/ synphilin-1 interaction and inclusion body formation. J. Biol. Chem. 279(8):6834-6839.

Akten, B., et al. (2003) A role for CK2 in the Drosophila circadian oscillator. Nat. Neurosci. 6(3):251-257.

Kawaguchi, Y., et al. (2003) Conserved protein kinases encoded by herpesviruses and cellular protein kinase cdc2 target the same phosphorylation site in eukaryotic elongation factor 1delta. J. Virol. 77(4):2359-2368.

Provost, E., et al. (2003) Functional correlates of mutations in beta-catenin exon 3 phosphorylation sites. J. Biol. Chem. 278(34):31781-31789.

D.W. Litchfield (2003) Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. Biochem. J. 369(Pt 1):1-15. Review.

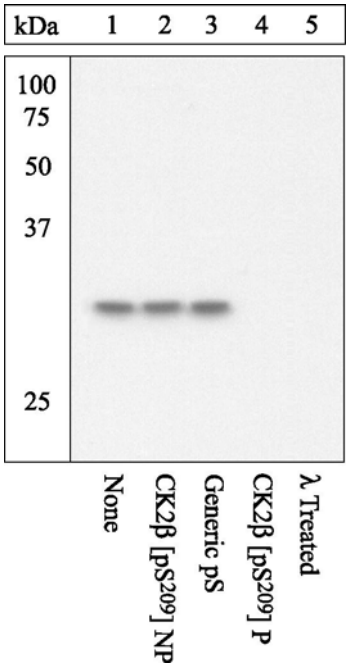
Meggio, F. and L.A. Pinna (2003) One-thousand-and-one substrates of protein kinase CK2? FASEB J. 17(3):349-368. Review.

Sarno, S., et al. (2002) Toward the rational design of protein kinase casein kinase-2 inhibitors. Pharmacol. Ther. 93(2-3):159-168. Review.

Litchfield, D.W., et al. (1995) The protein kinase from mitotic human cells that phosphorylates Ser-209 on the casein kinase II beta-subunit is p34cdc2. Biochim. Biophys. Acta. 1269(1):69-78.

www.invitrogen.com

Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com



Antibody-Peptide Competition and Phosphatase Treatment

Extracts of HeLa cells were resolved by SDS-PAGE on a 14% Tris-glycine gel and transferred to PVDF. The membrane was either left untreated (1-4) or treated with lambda (λ) phosphatase (5), blocked with a 3% Milk-TBST buffer for one hour at room temperature, and then incubated with the CK2β [pS^{209}] antibody for two hours at room temperature in a 3% Milk-TBST buffer, following prior incubation with: no peptide (1, 5), the non-phosphopeptide corresponding to the phosphopeptide immunogen (2), a generic phosphoserine-containing peptide (3), or the phosphopeptide immunogen (4). 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 only the phosphopeptide corresponding to CK2β [pS^{209}] blocks the signal, demonstrating the specificity of the antibody. The data also show that phosphatase stripping eliminates the signal, further verifying that the antibody is phospho-specific.

www.invitrogen.com
Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com

PI441090G (Rev 11/08) DCC-08-1089

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

www.invitrogen.com
Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com

PI441090G (Rev 11/08) DCC-08-1089