

Phospho-CrkII (Tyr221) Antibody

100 µl
 (10 western blots)

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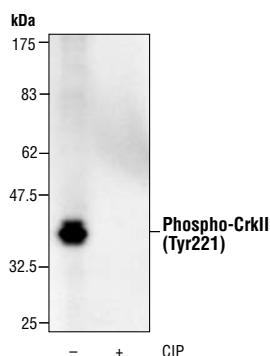
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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Entrez-Gene ID #1398
Swiss-Prot Acc. #P46108

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, Hm	42 kDa	Rabbit**

Background: CrkII, a cellular homologue of v-Crk, belongs to a family of adaptor proteins with an SH2-SH3-SH3 domain structure that transmits signals from tyrosine kinases (1). The primary function of Crk is to recruit cytoplasmic proteins in the vicinity of tyrosine kinases through SH2-phospho-tyrosine interaction. Thus, the output from Crk depends on the SH3-binding proteins, which include the C3G and Sos guanine nucleotide exchange proteins, Abl tyrosine kinase, DOCK180 and some STE20-related kinases. The variety of Crk-binding proteins indicates the pleiotropic function of Crk (2). The two CrkII SH3 domains are separated by a 54 amino acid linker region, which is highly conserved in *Xenopus*, chicken and mammalian CrkII proteins (3). Tyrosine 221 in this region is phosphorylated by the Abl tyrosine kinase (4), IGF-1 receptor (5) and EGF receptor (6). Once Tyr221 is phosphorylated, CrkII undergoes a change in intramolecular folding and SH2-pTyr interaction, which causes rapid dissociation of CrkII from the tyrosine kinase complex (3).



Western blot analysis of extracts from K562 cells, untreated or calf intestinal phosphatase (CIP)-treated using Phospho-CrkII (Tyr221) Antibody.

Specificity/Sensitivity: Phospho-CrkII (Tyr221) Antibody detects endogenous levels of CrkII only when phosphorylated at Tyr221. The antibody cross-reacts with Tyr207-phosphorylated CrkL but does not cross-react with other tyrosine-phosphorylated proteins.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr221 of human CrkII. Antibodies are purified by protein A and peptide affinity chromatography.

Background References:

- (1) Zvara, A. et al. (2001) *Oncogene* 20, 951–961.
- (2) Kiyokawa, E. et al. (1997) *Crit. Rev. Oncog.* 8, 329–342.
- (3) Rosen, S.K. et al. (1995) *Nature* 374, 477–479.
- (4) Amoui, M. and Miller, W.T. (2000) *Cell. Signal.* 12, 637–643.
- (5) Koval, A.P. et al. (1998) *Biochem. J.* 330, 923–932.
- (6) Hashimoto, Y. et al. (1998) *J. Biol. Chem.* 273, 17186–17191.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western Blotting 1:1000

For application specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.