

Phospho-CrkII (Tyr221) Antibody



Cell Signaling
TECHNOLOGY®

✓ 100 µl
(10 Western mini-blot)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications W	Species Cross-Reactivity H, Hm (M, R)	Molecular Wt. 42 kDa	Source Rabbit
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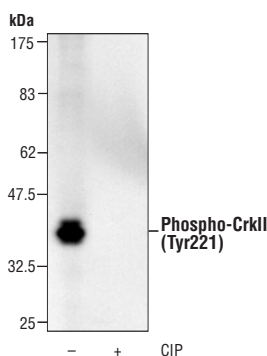
Background: CrkII, a cellular homologue of v-Crk, belongs to a family of adaptor proteins with an SH2-SH3-SH3 domain structure that transmits signals from tyrosine kinases (1). The primary function of Crk is to recruit cytoplasmic proteins in the vicinity of tyrosine kinases through SH2-phospho-tyrosine 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-I 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).

Specificity/Sensitivity: Phospho-CrkII (Tyr221) Antibody detects endogenous levels of CrkII only when phosphorylated at tyrosine 221. 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 rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Tyr221 of human CrkII. Antibodies are purified by protein A and peptide affinity chromatography.

Selected Application References:

Burton, E.A. et al. (2003) Abl tyrosine kinases are required for infection by *Shigella flexneri*. *EMBO J.* 22, 5471–5479. Applications: W.



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

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.

Recommended Antibody Dilutions:

Western blotting 1:1000

Companion Products:

Phototope®-HRP Western Detection System:
Anti-rabbit IgG, HRP-linked Antibody #7074

Prestained Protein Marker, Broad Range (Premixed Format) #7720

Biotinylated Protein Ladder Detection Pack #7727

LumiGLO® Reagent and Peroxide #7003

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

Western Immunoblotting Protocol

For Western blots, incubate membrane with diluted anti-body in 5% w/v nonfat dry milk, 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.

- A1. ■ 1X Phosphate Buffered Saline (PBS)
- A2. ■ 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
- A3. ■ Transfer Buffer:
25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- A4. ■ 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).
- A5. ■ Nonfat Dry Milk (weight to volume [w/v])
- A6. ■ 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%).
- A7. ■ Wash Buffer:
1X TBS, 0.1% Tween-20 (TBS/T)
- A8. ■ Bovine Serum Albumin (BSA)
- A9. ■ Primary Antibody Dilution Buffer:
1X TBS, 0.1% Tween-20 with 5% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 µl Tween-20 (100%).
- A10. ■ Phototope®-HRP Western Blot Detection System #7072:
Includes biotinylated protein ladder, secondary anti-mouse (#7076) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
- A11. ■ Prestained Protein Marker, Broad Range (Premixed Format) #7720
- A12. ■ Biotinylated Protein Ladder Detection Pack #7727
- A13. ■ 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.

- B1. Treat cells by adding fresh media containing regulator for desired time.
- B2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- B3. 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.
- B4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- B5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- B6. Microcentrifuge for 5 minutes.
- B7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

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

- B8. 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.

- C1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- C2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- C3. Wash three times for 5 minutes each with 15 ml of TBS/T.
- C4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- C5. Wash three times for 5 minutes each with 15 ml of TBS/T.
- C6. 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.
- C7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D. Detection of Proteins

- D1. 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.

- D2. 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.