Phospho-Ezrin (Tyr353) Antibody

#3144 Store at -20°C

☑ 100 µl
(10 Western mini-blots)

Cell Signaling TECHNOLOGY®

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rev. 10/16/07

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W	H	80 kDa	Rabbit

Background: The ezrin, radixin and moesin (ERM) proteins function as linkers between the plasma membrane and the actin cytoskeleton and are involved in cell adhesion, membrane ruffling and microvilli formation (1). ERM proteins undergo intra or intermolecular interaction between their amino- and carboxy terminal domains, existing as inactive cytosolic monomers or dimers (2). Phosphorylation at a carboxy terminal threonine residue (Thr567 of ezrin, Thr564 of radixin, Thr558 of moesin) disrupts their amino- and carboxy terminal association and may play a key role in regulating ERM protein conformation and function (3,4). Phosphorylation at Thr567 of ezrin is required for cytoskeletal rearrangements and oncogene-induced transformation (5). Ezrin is also phosphorylated at tyrosine residues upon growth factor stimulation. Phosphorylation of Tyr353 of ezrin transmits a survival signal during epithelial differentiation (6).

Specificity/Sensitivity: Phospho-Ezrin (Tyr353) Antibody detects endogenous levels of Ezrin only when phosphorylated at Tyr353. The antibody does not cross-react with phosphorylated Moesin or Radixin.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Tyr353 of human Ezrin. Antibodies are purified by protein A and peptide affinity chromatography.

Background References:

(1) Tsukita, S. and Yonemura, S. (1999) *J. Biol. Chem.* 274, 34507–34510.

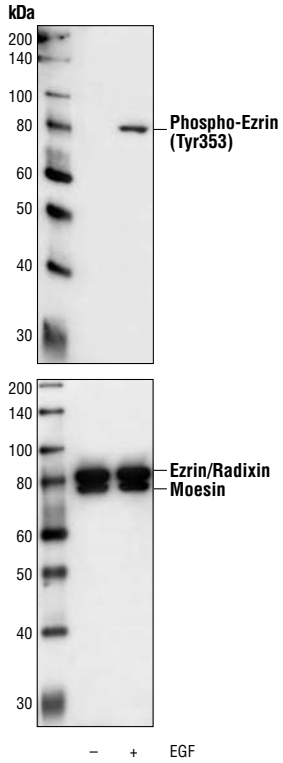
(2) Mangeat, P. et al. (1999) *Trends Cell Biol.* 9, 187–192.

(3) Matsui, T. et al. (1998) *J. Cell Biol.* 140, 647–657.

(4) Gautreau, A. et al. (2000) *J. Cell Biol.* 150, 193–203.

(5) Tran Quang, C. et al. (2000) *EMBO J.* 19, 4565–4576.

(6) Gautreau, A. et al. (1999) *Proc. Natl. Acad. Sci. USA* 96, 7300–7305.



Western blot analysis of extracts from A431 cells, untreated or EGF-treated using Phospho-Ezrin (Tyr353) Antibody (upper) or Ezrin/Radixin/Moesin Antibody #3142 (lower).

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.**

Recommended Antibody Dilutions:
Western Blotting 1:1000

Companion Products:

Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Antibody #3141

Ezrin/Radixin/Moesin Antibody #3142

Ezrin Antibody #3145

Moesin Antibody #3146

Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) (41A3) Rabbit mAb #3149

Moesin (Q480) Antibody #3150

Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071

Anti-rabbit IgG, HRP-linked Antibody #7074

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

Biotinylated Protein Ladder #7727

20X LumiGLO® Reagent and 20X Peroxide #7003

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.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **IC**—Immunocytochemistry **IF**—Immunofluorescence **F**—Flow cytometry **E**—ELISA **D**—DELFIA®

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **X**—Xenopus **Z**—zebra fish **B**—bovine **All**—all species expected

Species enclosed in parentheses are predicted to react based on 100% sequence homology.

#3144

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.

1. 1X Phosphate Buffered Saline (PBS)
2. **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
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **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).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **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%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **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%).
10. **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.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **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.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. 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.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. 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.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. 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.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. 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.

2. 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.