Revision 3

Store at -20C

#8354

Cofilin Activation Antibody Sampler Kit

1 Kit (7 x 20 microliters)

Cell Signaling TECHNOLOGY®

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Product Includes	Product #	Quantity	Mol. Wt	Isotype/Source
Cofilin (D3F9) XP® Rabbit mAb	5175	20 µl	19 kDa	Rabbit IgG
Phospho-Cofilin (Ser3) (77G2) Rabbit mAb	3313	20 µl	19 kDa	Rabbit IgG
LIMK2 (8C11) Rabbit mAb	3845	20 µl	70 kDa	Rabbit IgG
TESK1 (D49D4) Rabbit mAb	4655	20 µl	68 kDa	Rabbit IgG
ROCK1 (C8F7) Rabbit mAb	4035	20 µl	160 kDa	Rabbit
Chronophin/PDXP (C85E3) Rabbit mAb	4686	20 µl	31 kDa	Rabbit IgG
SSH1 (E1K3W) Rabbit mAb	13578	20 µl	140 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

Please visit cellsignal.com for individual component applications, species cross-reactivity, dilutions, protocols, and additional product information.

Description

The Cofilin Activation Antibody Sampler Kit provides an economical means to evaluate the presence and status of cofilin activation. The kit contains enough primary antibody to perform two western blot experiments per antibody.

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibodies.

Background

Cofilin and actin-depolymerization factor (ADF) are members of a family of essential conserved small actin-binding proteins that play pivotal roles in cytokinesis, endocytosis, embryonic development, stress response, and tissue regeneration (1). In response to stimuli, cofilin promotes the regeneration of actin filaments by severing preexisting filaments (2). The severing activity of cofilin is inhibited by LIMK or TESK phosphorylation at Ser3 of cofilin (3-5). Phosphorylation at Ser3 also regulates cofilin translocation from the nucleus to the cytoplasm (6).

LIM kinases (LIMK1 and LIMK2) are serine/threonine kinases that have two zinc finger motifs, known as LIM motifs, in their amino-terminal regulatory domains (7). LIM kinases are involved in actin cytoskeletal regulation downstream of Rho-family GTPases, PAKs, and ROCK (8,9). PAK1 and ROCK phosphorylate LIMK1 or LIMK2 at the conserved Thr508 or Thr505 residues in the activation loop, increasing LIMK activity (9-11). Activated LIM kinases inhibit the actin depolymerization activity of cofilin by phosphorylation at the amino-terminal Ser3 residue of cofilin (12,13).

Testis-specific kinase 1 (TESK1) is an LIMK-related protein kinase originally identified to be highly expressed in testes and subsequently shown to be expressed in a wide variety of tissues and cell types (14-17). TESK1 phosphorylates the actin severing protein cofilin at Ser3, inactivating cofilin and thus regulating the organization of the actin cytoskeleton (15). Integrin signaling activates TESK1 activity and leads to stress fiber formation and cell spreading (15,18,19). TESK1 is involved in regulation of ERK signaling through its interaction with Spry2 (20) and regulation of cell spreading through its interaction with the focal adhesion protein actopaxin/α-parvin (18).

Chronophin (CIN, PDXP) is a haloacid dehalogenase phosphatase that dephosphorylates cofilin. Alteration of CIN activity through overexpression of either the wildtype or phosphatase-inactive mutant CIN interferes with actin dynamics, cell morphology and cytokinesis (21).

ROCK (Rho-associated kinase), a family of serine/threonine kinases, is an important downstream target of GTPase Rho and plays an important role in Rho-mediated signaling. Two isoforms of ROCK have been identified (ROCK1 and ROCK2). ROCK is composed of N-terminal catalytic, coiled-coil, and C-terminal PH (pleckstrin homology) domains. The C-terminus of ROCK negatively regulates its kinase activity (22,23). Caspase-3-induced cleavage of ROCK1 and direct cleavage of ROCK2 by granzyme B (grB) activates ROCK and leads to phosphorylation of myosin light chain and inhibition of myosin phosphatase (24). This phosphorylation may account for the mechanism by which Rho regulates cytokinesis, cell motility, cell membrane blebbing during apoptosis, and smooth muscle contraction (25-27).

Slingshot homolog 1 (SSH1) can also dephosphorylate LIMK kinases, suppressing LIMK phosphorylation of cofilin (28). In addition, SSH1 modulates actin dynamics by stabilizing F-actin and promoting actin bundling independent of its cofilin phosphatase activity (29). SSH1 activity is regulated

by phosphorylation and protein-protein interaction through various signaling pathways (1). Binding of SSH1 to F-actin stimulates its cofilin phosphatase activity (30).

Background References

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