

PathScan® Total β -Actin Sandwich ELISA Antibody Pair



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✓ 1 Kit
(4 X 96 assays)

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For Research Use Only. Not For Use In Diagnostic Procedures.

Species Cross-Reactivity: H, R, M, Hm, Mk

Description: CST's PathScan® Total β -Actin Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Total β -Actin Sandwich ELISA Kit #7880. Capture and detection antibodies (100X stocks) and an HRP-linked secondary antibody (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The β -actin rabbit capture antibody is coated in PBS overnight onto a 96 well microplate. After blocking, cell lysate is added followed by pan-actin mouse detection antibody and HRP-linked, anti-mouse IgG antibody. HRP substrate (TMB) is then added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of β -actin.

Reagents not supplied:

Phosphate Buffered Saline (PBS-20X) #9808

Phosphate Buffered Saline with Tween®20 (PBST-20X) #9809

Cell Lysis Buffer (10X) #9803

TMB Substrate #7004

STOP Solution #7002

Blocking Buffer: 1X PBS/0.05% Tween®20, 1% BSA

96 Well Microplates**

Microplate Reader

** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592).

Notes: Antibody pairs have been optimized using recommended buffers, reagents, plates and the included protocol. Solutions should be made fresh daily.

Background: Actin, a ubiquitous protein in eukaryotes, is the major component of the cytoskeleton. At least six isoforms are known in mammals. Nonmuscle β - and γ -actin, also known as cytoplasmic actin, are predominantly expressed in nonmuscle cells, controlling cell structure and motility (1). α -cardiac and α -skeletal actin are expressed in striated cardiac and skeletal muscles, respectively; two smooth muscle actins, α - and γ -actin, are found primarily in vascular smooth muscle and enteric smooth muscle, respectively. These actin isoforms regulate contractile potentials for muscle cells (1). Actin exists mainly as a fibrous polymer, F-actin. In response to cytoskeletal reorganizing signals during processes such as cytokinesis, endocytosis, or stress, cofilin promotes fragmentation and depolymerization of F-actin, resulting in an increase in the monomeric globular form, G-actin (2). The Arp2/3 complex stabilizes

Product Includes	Item #	Volume	Cap Color	Storage Temp
β -Actin Capture Rabbit mAb (100X)	76027	400 μ L	Pink	4°C
Pan-Actin Detection Mouse mAb (100X)	46616	400 μ L	Blue	4°C
Anti-Mouse IgG, HRP-Linked Antibody (1000X)	20725	40 μ L	Yellow	-20°C

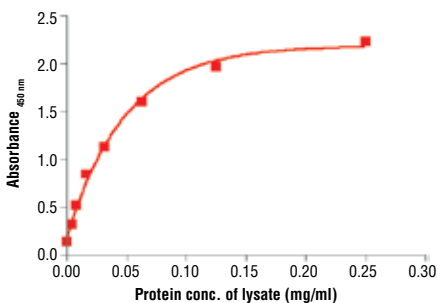


Figure 1. The relationship between the protein concentration of the lysate from HeLa cells and the absorbance at 450 nm using the PathScan® Total β -Actin Sandwich ELISA Antibody Pair is shown.

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F-actin fragments and promotes formation of new actin filaments (2). It has been reported that actin is hyperphosphorylated in primary breast tumors (3). Cleavage of actin under apoptotic conditions has been observed *in vitro* and in cardiac and skeletal muscle (4-6). Actin cleavage by caspase-3 may accelerate ubiquitin/proteasome dependent muscle proteolysis (6).

Background References:

- (1) Herman, I.M. (1993) *Curr. Opin. Cell Biol.* 5, 48-55.
- (2) Condeelis, J. (2001) *Trends Cell Biol.* 11, 288-293.
- (3) Lim, Y.P. et al. (2004) *Clin. Cancer Res.* 10, 3980-3987.
- (4) Kayalar, C. et al. (1996) *Proc. Natl. Acad. Sci. USA.* 93, 2234-2238.
- (5) Communal, C. et al. (2002) *Proc. Natl. Acad. Sci. USA.* 99, 6252-6256.
- (6) Du, J. et al. (2004) *J. Clin. Invest.* 113, 115-123.

U.S. Patent No. 5,675,063
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Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide

Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebra fish B—bovine

Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

PathScan® Sandwich ELISA Antibody Pair Protocol

A Required Reagents

- Coating Buffer:** 1X PBS, (20X PBS #9808)
3.2 mM Na₂HPO₄, 0.5 mM KH₂PO₄, 1.3 mM KCl, 135 mM NaCl, pH 7.4
- Wash Buffer:** 1X PBS/0.05% Tween®20, (20X PBST #9809)
- Blocking Buffer:** 1X PBS/0.05% Tween®20, 1% BSA
- 1X Cell Lysis Buffer:** (10X Cell Lysis Buffer #9803)
20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM ethylene diamine tetraacetate (EDTA),
1 mM ethylene glycol-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA),
1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate,
1 mM Na₃VO₄, 1 μg/ml leupeptin.
- TMB Substrate:** (TMB Substrate #7004)
- STOP Solution:** (STOP Solution #7002)

NOTE: Reagents should be made fresh daily

B Coating Procedure

- Rinse microplate with dH₂O. Add 200 μl of dH₂O and discard liquid. Blot on paper towel to make sure wells are dry.
- Dilute capture antibody 1:100 in PBS. For a single 96 well plate, add 100 μl of Capture Antibody Stock to 9.9 ml PBS. Mix well and add 100 μl/well. Cover plate and incubate overnight at 4°C (17-20 hours).
- After overnight coating, gently uncover plate and wash wells:**
 - Discard plate contents into a receptacle.
 - Wash 4 times with Wash Buffer, 200 μl each time for each well. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
 - Clean the underside of all wells with a lint-free tissue.
- Block plates. Add 150 μl of Blocking Buffer/well, cover plate and incubate at 37°C for 2 hours.
- After blocking, wash plate as in Step 3. Plate is ready to use.

C Preparing Cell Lysates

- Aspirate media, treat cells by adding fresh media containing regulator for desired time.
- To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
- Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm diameter plate) and incubate the plate on ice for 5 minutes.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- Microcentrifuge for 10 minutes at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at -80°C in single-use aliquots.

D Test Procedure

- Lysates can be used undiluted or diluted in Blocking Buffer. 100 μl of lysate is added per well. Cover plate and incubate at 37°C for 2 hours.
- Wash plate as in Coating Procedure, Step 3.
- Dilute detection antibody 1:100 in Blocking Buffer. For a single 96 well plate, add 100 μl of Detector Antibody Stock to 9.9 ml of Blocking Buffer. Mix well and add 100 μl/well. Cover plate and incubate at 37°C for 1 hour.
- Plate is washed as in Coating Procedure, Step 3.
- Secondary antibody, either, anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in Blocking Buffer. For a single 96 well plate, add 10 μl of secondary antibody stock to 9.99 ml of Blocking Buffer. Mix well and add 100 μl/well. Cover and incubate at 37°C for 30 minutes.
- Wash plate as in Coating Procedure, Step 3.
- Add 100 μl of TMB Substrate per well. Cover and incubate at 37°C for 10 minutes.
- Add 100 μl of STOP Solution per well.
- Read plate on a microplate reader at Absorbance 450 nm.