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## PathScan® Total $\beta$ -Actin Sandwich ELISA Kit

1 Kit (96 assays)

**Species Cross Reactivity:** H M R Hm Mk  
**UniProt ID:** #P60709  
**Entrez-Gene Id:** #60

**For Research Use Only. Not for Use in Diagnostic Procedures.**

Product Includes	Product #	Quantity	Color	Storage Temp
$\beta$ -Actin Rabbit mAb Coated Microwells	74040	96 tests		+4C
$\beta$ -Actin Mouse Detection mAb	23722	1 ea	Green (Lyophilized)	+4C
Anti-mouse IgG, HRP-linked Antibody (ELISA Formulated)	13304	1 ea	Red (Lyophilized)	+4C
Detection Antibody Diluent	13339	11 ml	Green	+4C
HRP Diluent	13515	11 ml	Red	+4C
TMB Substrate	7004	11 ml		+4C
STOP Solution	7002	11 ml		+4C
Sealing Tape	54503	2 ea		+4C
ELISA Wash Buffer (20X)	9801	25 ml		+4C
ELISA Sample Diluent	11083	25 ml	Blue	+4C
Cell Lysis Buffer (10X)	9803	15 ml		-20C

Kit contents scale proportionally with size, except sealing tape.

Example: The V1 kit contains 5X the listed quantities above, but will exclude the sealing tape.

The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

### Description

The PathScan® Total  $\beta$ -Actin Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of  $\beta$ -actin. A  $\beta$ -actin rabbit antibody has been coated onto the microwells. After incubation with cell lysates,  $\beta$ -actin is captured by the coated antibody. Following extensive washing, a  $\beta$ -actin mouse detection antibody is added to detect the captured  $\beta$ -actin. An anti-mouse IgG, HRP-linked antibody is then used to recognize the bound detection antibody. HRP substrate (TMB) is added to develop color. The magnitude of the absorbance for this developed color is proportional to the quantity of  $\beta$ -actin.

\*Antibodies in this kit are custom formulations specific to kit.

### Specificity/Sensitivity

CST's PathScan® Total  $\beta$ -Actin Sandwich ELISA Kit detects endogenous levels of  $\beta$ -actin. As shown in Figure 1,  $\beta$ -actin is readily detected in HeLa cells using the PathScan® Total  $\beta$ -Actin Sandwich ELISA Kit. Total levels of  $\beta$ -actin remain unchanged after IFN- $\alpha$  treatment as shown by western analysis. The PathScan® Total  $\beta$ -Actin Sandwich ELISA Kit does not cross-react with  $\alpha$ -smooth muscle actin,  $\alpha$ -sarcomeric muscle actin or  $\gamma$ -actin. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

### Background

Actin, a ubiquitous eukaryotic protein, is the major component of the cytoskeleton. At least six isoforms are known in mammals. Nonmuscle  $\beta$ - and  $\gamma$ -actin, also known as cytoplasmic actin, are ubiquitously expressed, controlling cell structure and motility (1). While all actin isoforms are highly homologous, cytoplasmic  $\beta$ - and  $\gamma$ -actin protein sequences differ by only four biochemically similar amino acids (2). For this reason, antibodies raised to  $\beta$ -actin may cross-react with  $\gamma$ -actin, and vice versa.  $\alpha$ -cardiac and  $\alpha$ -skeletal actin are expressed in striated cardiac and skeletal muscles, respectively; two smooth muscle actins,  $\alpha$ - and  $\gamma$ -actin, are found primarily in vascular smooth muscle and enteric smooth muscle, respectively. These actin isoforms regulate the contractile potential of 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 (3). The ARP2/3 complex stabilizes F-actin fragments and promotes formation of new actin filaments (3). Research studies have shown that actin is hyperphosphorylated in primary breast tumors (4). Cleavage of actin under apoptotic conditions has been observed *in vitro* and in cardiac and skeletal muscle, as shown in research studies (5-7). Actin cleavage by caspase-3 may accelerate ubiquitin/proteasome-dependent

muscle proteolysis (7).

## Background References

1. Herman, I.M. (1993) *Curr. Opin. Cell Biol.* 5, 48-55.
  2. Perrin, B.J. and Ervasti, J.M. (2010) *Cytoskeleton (Hoboken)* 67, 630-4.
  3. Condeelis, J. (2001) *Trends Cell Biol* 11, 288-93.
  4. Lim, Y.P. et al. (2004) *Clin Cancer Res* 10, 3980-7.
  5. Kayalar, C. et al. (1996) *Proc Natl Acad Sci U S A* 93, 2234-8.
  6. Communal, C. et al. (2002) *Proc Natl Acad Sci U S A* 99, 6252-6.
  7. Du, J. et al. (2004) *J Clin Invest* 113, 115-23.
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