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#77397

# TGF- $\beta$ Fibrosis Pathway Antibody Sampler Kit



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

Products Included	Product #	Quantity	Mol. Wt.	Isotype/Source
$\alpha$ -Smooth Muscle Actin (D4K9N) XP <sup>®</sup> Rabbit mAb	19245	20 $\mu$ l	42 kDa	Rabbit IgG
COL1A1 (E8I9Z) Rabbit mAb	91144	20 $\mu$ l	220 kDa	Rabbit IgG
Smad2/3 (D7G7) XP <sup>®</sup> Rabbit mAb	8685	20 $\mu$ l	52, 60 kDa	Rabbit IgG
Smad2 (D43B4) XP <sup>®</sup> Rabbit mAb	5339	20 $\mu$ l	60 kDa	Rabbit IgG
P-SMAD2 (S465/S467) (E8F3R) Rabbit mAb	18338	20 $\mu$ l	60 kDa	Rabbit IgG
YKL-40 (E2L1M) Rabbit mAb	47066	20 $\mu$ l	30-40 kDa	Rabbit IgG
P-Smad2 (S465/467)/Smad3 (S423/425) (D27F4) Rabbit mAb	8828	20 $\mu$ l	52, 60 kDa	Rabbit IgG
TGF- $\beta$ (56E4) Rabbit mAb	3709	20 $\mu$ l	12, 45-60 kDa	Rabbit IgG
TGF- $\beta$ Receptor II Antibody	79424	20 $\mu$ l	85 kDa	Rabbit
Anti-rabbit IgG, HRP-linked Antibody	7074	100 $\mu$ l		Goat

See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Description:** The TGF- $\beta$  Fibrosis Pathway Antibody Sampler Kit provides an economical means of investigating activation of TGF- $\beta$ /SMAD2/3 signaling pathways in cells or tissues that lead to the expression of profibrotic genes, including expression of  $\alpha$ -Smooth Muscle Actin in activated fibroblasts, and upregulation of Collagen1A1, Col11A1, and YKL-40. The kit includes enough antibodies to perform at least two western blot experiments with each primary antibody.

**Background:** Transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily members are critical regulators of cell proliferation and differentiation, developmental patterning and morphogenesis, and disease pathogenesis (1-4). In the context of fibrosis, TGF- $\beta$  signaling to SMAD2/3 is one of the biggest drivers of the profibrotic program (5).

TGF- $\beta$  elicits signaling through three cell surface receptors: type I (RI), type II (RII), and type III (RIII). In response to ligand binding, the type II receptors form stable heterotrimeric complexes with the type I receptors, allowing phosphorylation and activation of type I receptor kinase. Activated type I receptors associate with SMAD2/3 and phosphorylate them on a conserved carboxy terminal SSXS motif. The phosphorylated SMADs dissociate from the receptor and form a heterotrimeric complex with the co-Smad (Smad4), allowing translocation of the complex to the nucleus. Once in the nucleus, phosphorylated SMAD2/3 targets a subset of DNA binding proteins to regulate the transcriptional program (6-8).

In the context of fibrosis, SMAD2/3 activation upregulates expression of profibrotic genes such as COL1A1 and other ECM modulators that modify the extracellular matrix of the tissue. (9). TGF- $\beta$ /SMAD2/3 signaling also induces expression of  $\alpha$ -Smooth Muscle Actin in fibroblasts, causing transformation of these cells to myofibroblasts (10). Myofibroblasts

further modify the ECM, causing excessive accumulation of collagens and other ECM components. Injury to the tissue attracts macrophages and other immune cells and the fibrotic tissue soon becomes a site of inflammation (11). In this profibrotic, pro-inflammatory environment, YKL-40, also known as Chitinase-3-like protein 1 (CHI3L1), is secreted. YKL-40 is a pro-inflammatory glycoprotein that also contributes to the progression of fibrosis (12). Measurement of collagen content,  $\alpha$ -Smooth Muscle Actin, and the release of YKL-40 are predictive of fibrotic activity.

**Specificity/Sensitivity:**  $\alpha$ -Smooth Muscle Actin (D4K9N) XP<sup>®</sup> Rabbit mAb recognizes endogenous levels of total  $\alpha$ -smooth muscle protein. COL1A1 (E8I9Z) Rabbit mAb recognizes endogenous levels of total COL1A1 protein. Smad2/3 (D7G7) XP<sup>®</sup> Rabbit mAb recognizes endogenous levels of total Smad2/3 protein. Smad2 (D43B4) XP<sup>®</sup> Rabbit mAb detects endogenous levels of total Smad2 protein. This antibody does not cross-react with Smad3. Phospho-Smad2 (Ser465/Ser467) (E8F3R) Rabbit mAb recognizes endogenous levels of Smad2 protein when phosphorylated at Ser465 and Ser467. YKL-40 (E2L1M) Rabbit mAb recognizes endogenous levels of total YKL-40 protein. Phospho-Smad2 (Ser465/467)/Smad3 (Ser423/425) (D27F4) Rabbit mAb recognizes endogenous levels of Smad2 protein when phosphorylated at Ser465 and Ser467. This antibody also recognizes endogenous levels of Smad3 protein when phosphorylated at Ser422 only or at both Ser423 and Ser425. TGF- $\beta$  (56E4) Rabbit mAb detects recombinant TGF- $\beta$ 1 and TGF- $\beta$ 3 proteins. The antibody also detects endogenous levels of the TGF- $\beta$  precursor proteins. TGF- $\beta$  Receptor II Antibody recognizes endogenous levels of total TGF- $\beta$  Receptor II protein.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

Please visit [www.cellsignal.com](http://www.cellsignal.com) for validation data and a complete listing of recommended companion products.

#### Background References:

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- (2) de Caestecker, M.P. et al. (2000) *J Natl Cancer Inst* 92, 1388-402.
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- (6) Wu, G. et al. (2000) *Science* 287, 92-7.
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- (9) Bagalad, B.S. et al. *J Oral Maxillofac Pathol* 21, 462-3.
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- (11) Johansen, J.S. (2006) *Dan Med Bull* 53, 172-209.

residues near the amino terminus of human  $\alpha$ -Smooth Muscle Actin protein. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Phe1197 of human COL1A1 protein. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding His198 of human Smad2/3 protein. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the amino terminus of mouse Smad2 protein. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser465/467 of human Smad2 protein. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the amino terminus of human YKL-40 protein. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser465/467 of human Smad2 protein. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to a region in the carboxy terminus of TGF- $\beta$ 1 protein. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Asp247 of human TGF- $\beta$  Receptor II protein. Antibodies are purified by protein A and peptide affinity chromatography.

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