ELISA*ONE*™ β-CATENIN Assays

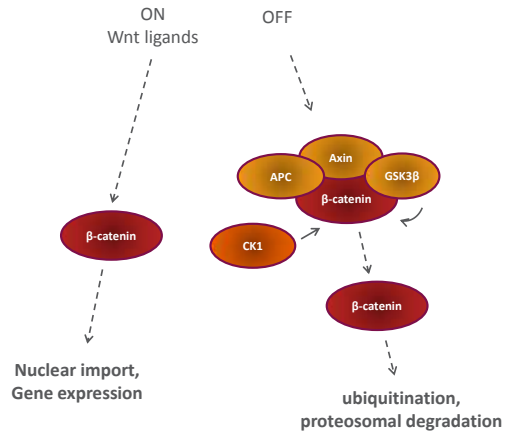
ELISA*ONE*™ assay kits for analysis of cellular β-CATENIN (p-Ser45) phosphorylation

ELISA*ONE*™ Overview

ELISA*ONE* assay kits are for the detection of cellular proteins. A whole new way of performing cellular assays, ELISA*ONE* takes the hard work out of running a standard ELISA, while still giving the high quality results expected from a sandwich immunoassay. Fully self-contained kits are supplied in a convenient 96-well format. Simple to use and highly sensitive, ELISA*ONE* kits are designed to get results, fast.

ELISA*ONE*™ phospho-β-catenin and Total β-catenin assays

β-catenin is part of a complex of proteins that constitute adherens junctions, and play an important role mediating cellular adhesion. β-catenin is also a key downstream effector in the Wnt signaling pathway.

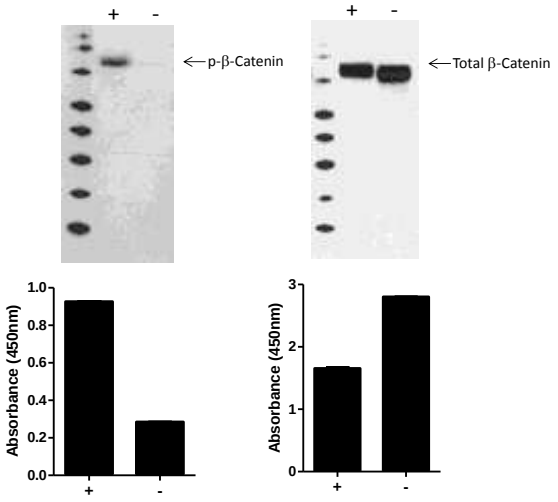


CK1 phosphorylates β-catenin on Ser45, which primes β-catenin for subsequent phosphorylation by GSK3β at Ser33, Ser37 and Thr41. Phosphorylation by GSK3β destabilizes β-catenin and targets it for ubiquitination and subsequent proteolytic degradation. β-catenin has also been found in complexes with the tumor suppressor protein APC. Mutations in APC are a cause of several cancer types, including colorectal cancer, pilomatrixoma, medulloblastoma and ovarian cancer.

ELISA*ONE*™ β-catenin (p-Ser45) assay technical specifications

Specificity:

TGR's ELISA*ONE* β-catenin (p-Ser45) assay kits detect endogenous levels of β-catenin (GenBank Accession NP_001895.1) in cellular lysates. Phospho-β-catenin assay kits only detect β-catenin when phosphorylated at Ser45. Total β-Catenin assay kits detect β-catenin irrespective of phosphorylation status. As shown, using the β-catenin assay kits or Western blot, β-catenin phosphorylation at Ser45 is detected in PMA/calyculin-treated A549 cells (+), compared with untreated A549 cells (-), consistent with Western blot results.



Species cross-reactivity:

Tested: Human, Mouse

Expected: Rat

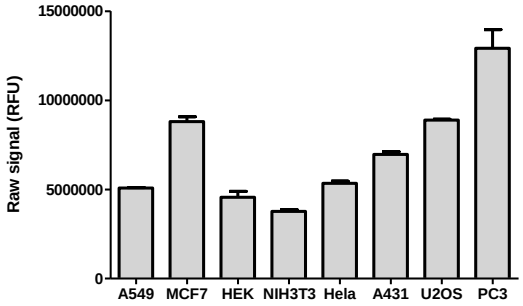
Other species should be tested on a case-by-case basis.

QC:

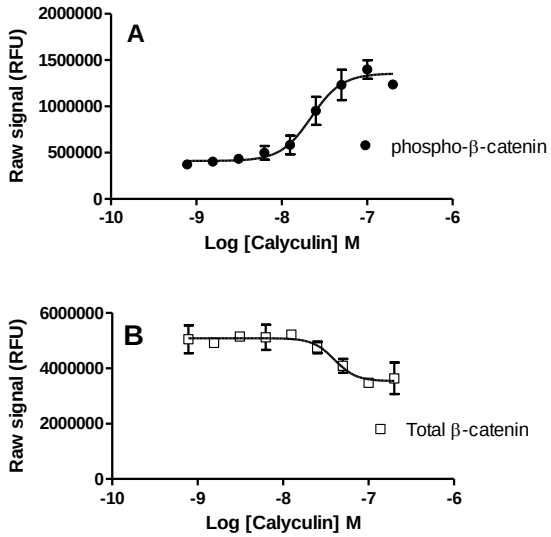
Phospho-β-catenin and total β-catenin assays are routinely tested against PMA-treated A549 cellular lysates. See certificate of analysis for Lot-specific information. Available at www.tgrbio.com.

ELISA*ONE*™ β-catenin assay performance

Various cell lines were seeded at 40k/mL in a 96 well plate in medium containing 10% FBS, and cultured overnight. The following day, cells were lysed with 120 µL/well of freshly prepared ELISA*ONE* Lysis Mix with shaking for 10 mins at room temperature. 50 µL of lysate was transferred to replicate wells of an ELISA*ONE* assay plate, and Antibody Mix specific for total β-catenin (50 µL/well) was added to the lysates. The plates were incubated for 1 hr at room temp, with shaking. Plates were washed and Substrate Mix was added. The plates were covered in foil and incubated for 10 min with shaking. Signal in the wells was determined using a Flexstation (Molecular Devices) plate reader. The results show that β-catenin is readily detectable in several human cell lines.



ELISA*ONE* β-*catenin assay kits are optimized for the rapid detection of* β-*catenin in cellular lysates.*



C2C12 cells were seeded at 5K cells/well in a 96 well plate containing 10% FBS, and cultured overnight. The following day cells were treated with various concentrations of calyculin A for 30 mins. Following treatment, the medium was removed and cells were lysed with 120 µL/well of freshly prepared ELISA*ONE* Lysis Mix with shaking for 10 mins at room temperature. 50 µL of lysate was transferred to replicate wells of an ELISA*ONE* assay plate, and 50 µL/well Antibody Mix specific for either (A) phospho-β-catenin (p-Ser45) or (B) total β-catenin was added to the lysates. The plates were incubated for 1 hr at room temp, with shaking. Plates were washed and Substrate Mix was added. The plates were covered in foil and incubated for 10 min with shaking. Signal in the wells was determined using a Flexstation (Molecular Devices) plate reader. Phosphorylation-induced depletion of total β-catenin is readily apparent.

Ordering Information

ELISA*ONE* Assay Kits are available now from:

CEDARLANE (www.cedarlanelabs.com)

US: 1-800-721-1644
CANADA: 1-800-268-5058

AXXORA (www.axxora.com)

US: 1-858-550-8830
Email: axxora-usa@axxora.com

Product	Pack Size	Catalog
Phospho-β-Catenin (S45)	24 assays	ERS023
	96 assays	EKT023
Total β-Catenin	24 assays	ERS033
	96 assays	EKT033

* ELISA*ONE* assay microplates are not supplied with 24pt kits, and must be purchase separately.

Complementary Products

ELISA*ONE*™ assay kits are available for the following targets:

MAPK Signaling:
ERK 1/2, p38 MAPK, JNK/SAPK, c-JUN

AKT Signaling:
AKT 1/2/3 (p-Thr308), AKT 1/2/3 (p-Ser473), p70S6K, ribosomal protein S6, GSK3β, BAD

STAT Signaling:
STAT1, STAT3, STAT5

NF-κB Signaling:
NF-κB p65, I-κBα, IKKα

p53 Signaling:
p53

Wnt Signaling:
β-Catenin, GSK3β

TGFβ Signaling:
SMAD1, SMAD3

Protein normalization:
GAPDH

Also available separately:

Product	Pack Size	Catalog
ELISA*ONE*™ 96-well regular microplates	1 plate	EPF001
	5 plates	EPF002
ELISA*ONE*™ 96-well strip-well microplates	1 plate	EPL001
	5 plates	EPL002
5X Lysis Buffer	100mL	EBF001
Enhancer Solution	100mL	EBF002
10X Wash Buffer	500mL	EBF003

Check our website (www.tgrbio.com) for the latest information on target availability.