

ELISAONE™ GSK3 β Assays

ELISAONE™ assay kits for analysis of cellular GSK3 β (p-Ser9) phosphorylation

ELISAONE™ Overview

ELISAONE assay kits are for the detection of cellular proteins. A whole new way of performing cellular assays, ELISAONE 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, ELISAONE kits are designed to get results, fast.

ELISAONE™ phospho-GSK3 β and Total GSK3 β assays

Glycogen synthase kinase-3 (GSK3) was initially identified for its ability to regulate glycogen synthesis in response to insulin, through its capacity to phosphorylate and inactivate glycogen synthase. Expressed as two closely related isoforms - GSK3 α and GSK3 β , these kinases are now known to be involved in a more diverse array of signalling pathways.

GSK3 β is an important element of the PI3 kinase/AKT signalling pathway, where AKT-mediated phosphorylation at Ser9 negatively regulates GSK3 β kinase activity. GSK3 is also a component of the Wnt signalling pathway, required for developmental processes.

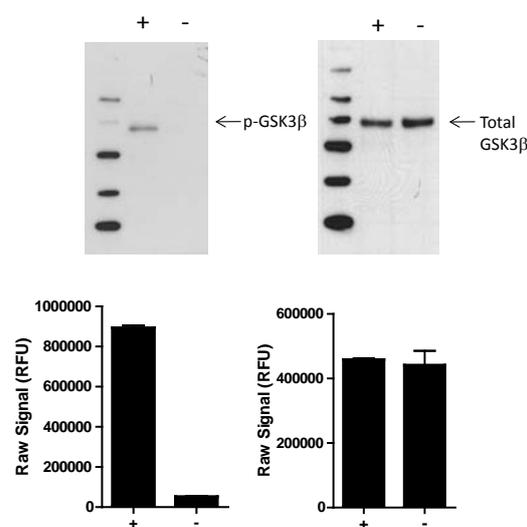
ELISAONE™ GSK3 β (p-Ser9) assay technical specifications

Specificity:

TGR's ELISAONE GSK3 β (p-Ser9) assay kits detect endogenous levels of GSK3 β (GenBank Accession NP_001139628) in cellular lysates. Phospho-GSK3 β assay kits only detect GSK3 β when phosphorylated at Ser9. Total GSK3 β assay kits detect GSK3 β irrespective of phosphorylation status.

As shown, using the GSK3 β assay kits or Western blot, GSK3 β phosphorylation at Ser9 is detected in

insulin-treated MCF7 cells (+), compared with MCF7 cells treated with PI3-kinase pathway inhibitors (-), whereas no change in total GSK3 β levels is observed.



Species cross-reactivity:

Tested: Human, Mouse

Expected: Rat

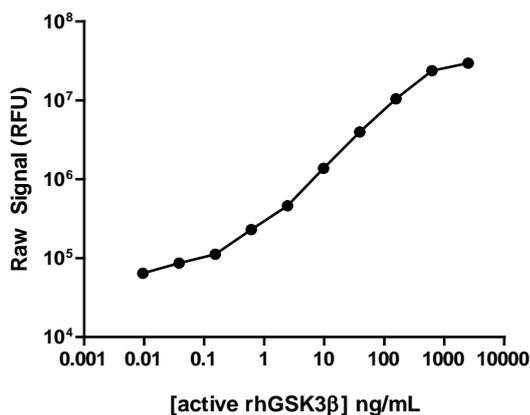
Other species should be tested on a case-by-case basis.

QC:

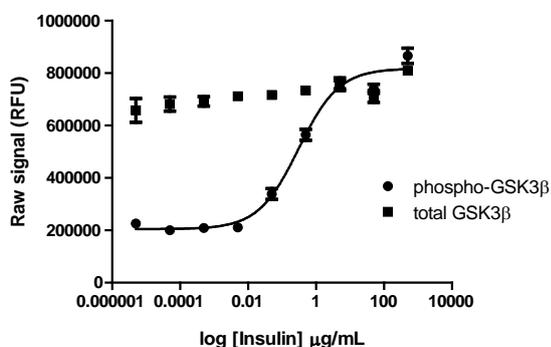
Phospho-GSK3 β and total GSK3 β assays are routinely tested against MCF7 cell lysates. See certificate of analysis for Lot-specific information. Available at www.tgrbio.com.

ELISAONE™ GSK3β assay performance

Recombinant human active GSK3β was prepared at various concentrations in Lysis Mix containing 0.1% BSA. 50 μL of these preparations were transferred to replicate wells of an ELISAONE assay plate, then Antibody Mix specific for phospho-GSK3β (50 μL/well) was added. 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 GSK3β is readily detectable to concentrations of around 100 pg/mL.



ELISAONE GSK3β assay kits are optimized for the rapid detection of GSK3β in cellular lysates.



NIH3T3 cells were seeded at 30K cells/well in medium containing 10% FBS in a 96 well microplate, and cultured overnight. The following day the culture medium was removed, and the cells were starved with serum-free medium for 60 mins, then stimulated with various concentrations of insulin for 15 min. The medium was removed from the wells, and the cells were lysed with 120 μL/well Lysis Mix, with shaking for 10 min. 50 μL of lysate was transferred to replicate wells of an ELISAONE assay plate, and Antibody Mix specific for either phospho GSK3β or total GSK3β (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.

Ordering Information

ELISAONE 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-GSK3β (S9)	24 assays	ERS026
	96 assays	EKT026
Total GSK3β	24 assays	ERS036
	96 assays	EKT036

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

Complementary Products

ELISAONE™ 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

*coming soon

Also available separately:

Product	Pack Size	Catalog
ELISAONE™ 96-wellregular microplates	1 plate	EPPF001
	5 plates	EPPF002
ELISAONE™ 96-wellstrip-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.