

ELISAONE™ p53 Assays

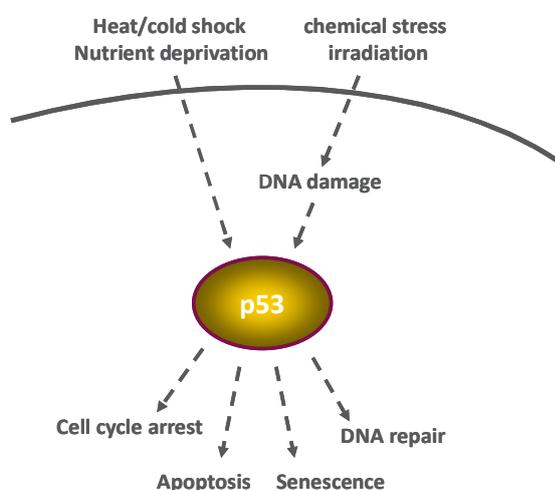
ELISAONE™ assay kits for analysis of cellular p53 phosphorylation

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-p53 and Total p53 assays

p53 (also known as protein 53 or tumor suppressor protein 53), is a transcription factor that plays a major role in regulating the cellular response to DNA damage and other replicative stresses. p53 becomes phosphorylated in response to several types of cellular stress, including DNA damage, oxidative stress and osmotic shock.



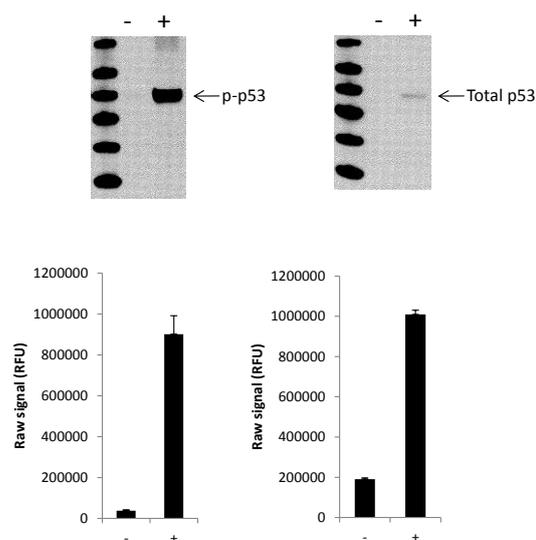
Phosphorylation of the N-terminus of p53, a region that contains a large number of phosphorylation sites, inhibits its negative regulator, MDM2, from interacting with p53. When phosphorylated, the half-life of the p53 protein is increased, leading to its accumulation in cells. Phosphorylated p53 also undergoes conformational change, allowing tetramerization. Activation of p53 can ultimately lead to either DNA repair, or programmed cell death.

ELISAONE™ p53 assay technical specifications

Specificity:

TGR's ELISAONE p53 assay kits detect endogenous levels of p53 (GenBank Accession NP_000537) in cellular lysates. Phospho-p53 assay kits detect p53 only when phosphorylated at Ser15. Total p53 assay kits detect p53 irrespective of phosphorylation status.

As shown below, using p53 assay kits, a significant increase in both (A) p53 phosphorylation at Ser15 and (B) accumulation of total p53, is detected in U-2 OS cells treated with doxorubicin for 18 hours, compared with untreated cells.



Species cross-reactivity:

Tested: Human

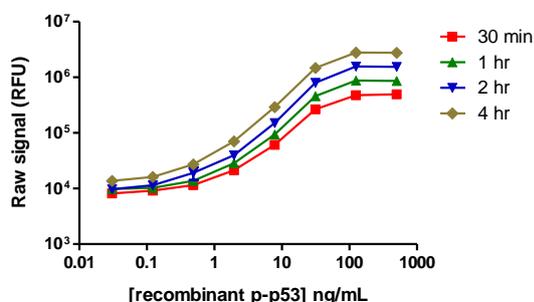
Other species should be tested on a case-by-case basis.

QC:

Phospho-p53 and Total-p53 assays are routinely tested against U-2 OS cellular lysates. See certificate of analysis for Lot-specific information. Available at www.tgrbio.com.

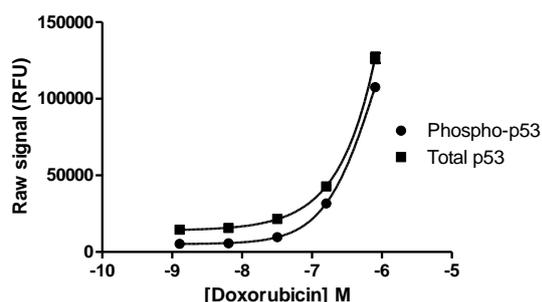
ELISAONE™ p53 assay performance

Using the ELISAONE phospho-p53 assay, the length of assay incubation time that was required to detect recombinant phospho-p53 was examined. After 30 mins, phospho-p53 concentrations of approximately 0.5 ng/mL were readily detected, while after 2 hour incubation, the limit of detection was approximately 0.2 ng/mL. Longer incubation times had little effect on overall limit of detection that was observed.



	30 min	1 hr	2 hr	4 hr
Limit of Detecton	0.5 ng/mL	0.5 ng/mL	0.2ng/mL	0.2 ng/mL

ELISAONE p53 assays are optimized for the rapid detection of p53 in cellular lysates.



U2OS Cells were seeded at 25K cells/well in a 96 well tissue culture plate. The following day, the media was aspirated and replaced with fresh media containing doxorubicin at various concentrations. The plate was incubated at 37°C for a further 18 hours. The media was removed from the wells, and the cells were lysed with 150 µL/well freshly prepared ELISAONE Lysis Mix, with shaking for 10 min at room temp. Lysates (50 µL) were transferred to replicate wells of an ELISAONE assay plate and analyzed for either phospho-p53, or Total p53 using the standard ELISAONE protocol. Briefly, Antibody Mix specific for either phospho-p53, or Total p53 was added to the lysates, and the plate was incubated for 1 hr at room temp, with shaking. The plate was 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 plate reader (Molecular Devices).

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-p53 (p-Ser15)	24 assays	ERS009
	96 assays	EKT009
Total p53	24 assays	ERS019
	96 assays	EKT019

* 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

AKT Signaling:

AKT 1/2/3, p70S6K

STAT Signaling:

STAT3, STAT5

NF-κB Signaling:

NF-κB p65, I-κBα

p53 Signaling:

p53

Protein normalization:

GAPDH

Also available separately:

Product	Pack Size	Catalog
ELISAONE™ 96-wellregular microplates	1 plate	EPF001
	5 plates	EPF002
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

More targets are being developed, including targets for interrogating apoptosis, WNT-signaling, and TGF-beta signaling. Check our website (www.tgrbio.com) for the latest information on target availability.