

ELISAONE™ STAT3 Assays

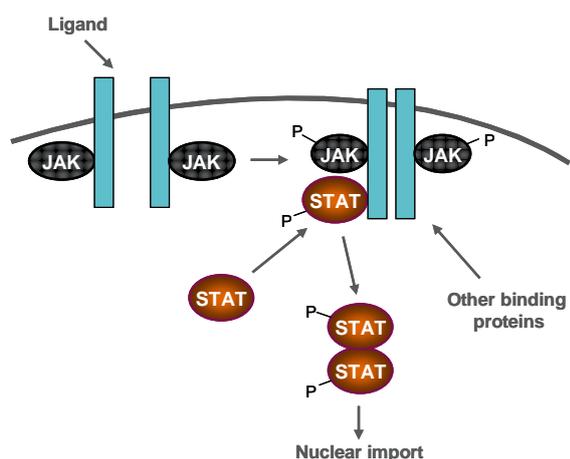
ELISAONE™ assay kits for analysis of cellular STAT3 phosphorylation

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-STAT3 and Total STAT3 assays

STAT3 phosphorylation was first described as an IL-6-mediated response, but has since been found to be induced by many stimuli. STAT3 exists as 2 splice variants (STAT3 α and STAT3 β), the expression of which appear cell type and cell maturation-dependent. STAT3 is phosphorylated at Tyr705 by activated JAK proteins, or by various receptor tyrosine kinases, which induces dimerization and translocation to the nucleus. STAT3 α (but not STAT3 β) is also phosphorylated at Ser727 in a MAPK and mTOR-dependent mechanism, which enhances transcriptional activity.



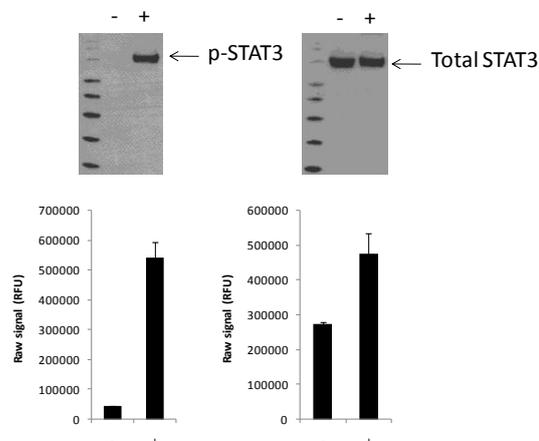
STAT3 has been linked to several processes important for tumour progression, and has been found to be constitutively active in certain tumour cells. Pro-oncogenic activities attributed to STAT3 include the upregulation of transcription of angiogenic proteins, and immune suppressive proteins.

ELISAONE™ STAT3 assay technical specifications

Specificity:

TGR's ELISAONE STAT3 assay kits detect endogenous levels of the 2 splice variants, STAT3 α and STAT3 β (Genbank Accessions NP_644805 and NP_998827) in cellular lysates. Phospho- STAT3 assay kits detect STAT3 only when phosphorylated at Tyr705. Total STAT3 assay kits detect STAT3 irrespective of phosphorylation status.

As shown below, using the STAT3 assay kits or Western blot, a significant stimulation of STAT3 phosphorylation at Tyr705 is detected in A431 cells treated with EGF (+) for 10 minutes compared with cells treated with AG1478 (-), which induced a reduction total STAT3 levels.



Species cross-reactivity:

Tested: Human

Expected: Mouse, Rat

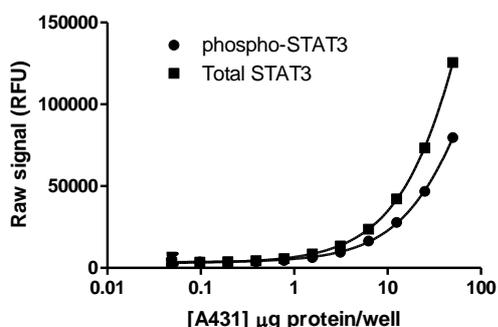
Other species should be tested on a case-by-case basis.

QC:

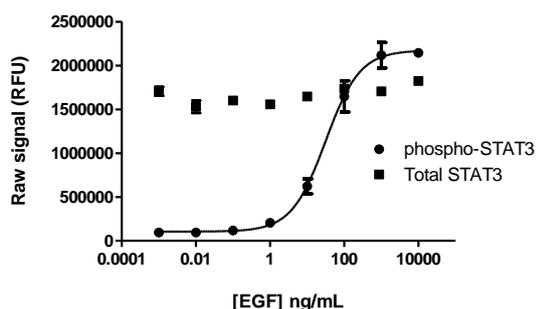
Phospho-STAT3 and Total STAT3 assays are routinely tested against A431 cellular lysates. See certificate of analysis for Lot-specific information. Available at www.tgrbio.com.

ELISAONE™ STAT3 assay performance

Using the ELISAONE phospho-STAT3 and Total STAT3 assays, the amount of lysate required to detect STAT3 in EGF-treated A431 cell lysates was examined. Up to 50 µg of lysate/well was loaded into replicate wells of an ELISAONE microplate, and analyzed for either phospho-STAT3 or total STAT3. Both phospho-STAT3 and total STAT3 were readily detected in less than 10 µg of lysate/well.



ELISAONE STAT3 assays are optimized for the rapid detection of STAT3 in cellular lysates.



Day 1: A431 cells were seeded at 25K/well in a 96 well tissue culture plate in medium containing 10% FBS.

Day 2: The cells were stimulated with various concentrations of EGF for 15 minutes. The media was removed, and the cells were lysed with 125 µL/well freshly prepared Lysis Mix, with shaking for 10 min. Lysates (50 µL) were transferred to replicate wells of an ELISAONE assay plate and analyzed for either phospho or total STAT3 using the standard ELISAONE protocol. Briefly, Antibody Mix specific for either phospho-STAT3, or Total STAT3 (50 µL/well), was added to the lysate, and the plate was incubated for 1 hr at room temp, with shaking. The plate was washed and Substrate Mix was added to the wells. The plate was 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-STAT3 (p-Tyr705)	24 assays	ERS005
	96 assays	EKT005
Total STAT3	24 assays	ERS015
	96 assays	EKT015

* ELISAONE assay microplates are not supplied with 24pt kits, but can 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.