

ELISAONE™ phospho-STAT5 Assay

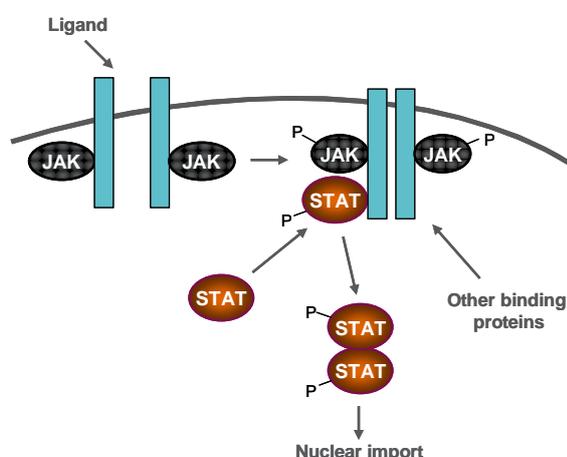
ELISAONE™ assay for analysis of cellular STAT5A/B phosphorylation

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-STAT5 assay

STAT5A and STAT5B (collectively known as STAT5) are two very closely related proteins. Although encoded by different genes, STAT5A and STAT5B share 96% identity at the protein level. Similarly to other STAT proteins, STAT5A and STAT5B are activated by tyrosine phosphorylation, usually by JAK proteins, at Tyr694 and Tyr699, respectively.



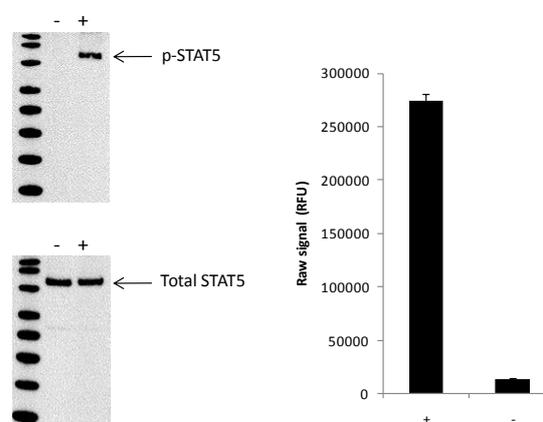
STAT5A and STAT5B show differential, cell-specific regulation, with STAT5A expression predominant in mammary tissue, while STAT5B expression is more abundant in muscle and liver tissues. STAT5 plays an integral role in immune cell development and regulation, and is an important mediator of IL-2 and IL-15 signaling in regulatory T cells.

ELISAONE™ phospho-STAT5 assay technical specifications

Specificity:

TGR's ELISAONE phospho-STAT5 assay kits detect endogenous levels of STAT5A and STAT5B (GenBank Accessions NP_003143 and NP_036580) in cellular lysates. Phospho-STAT5 assay kits detect STAT5A/B only when phosphorylated at Tyr694/699. A

As shown below, using either the phospho-STAT5 assay kit or Western blot, a significant stimulation of STAT5 phosphorylation at Tyr694/699 is detected in A431 cells treated with EGF for 10 minutes compared with cells treated with AG1478. Little change was detected in levels of total STAT5 in the same experiment.



Species cross-reactivity:

Tested: Human

Expected: Mouse, Rat

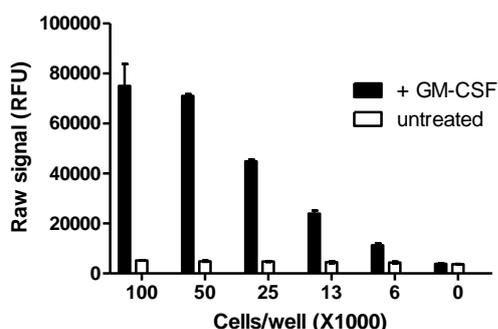
Other species should be tested on a case-by-case basis.

QC:

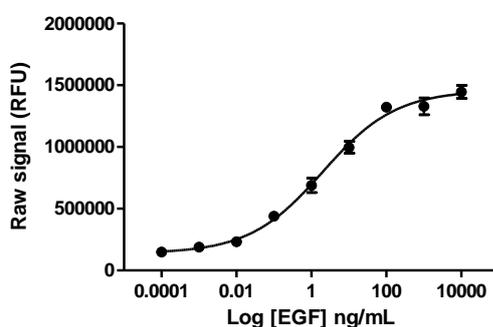
Phospho-STAT5 assays are routinely tested against A431 cellular lysates. See certificate of analysis for Lot-specific information. Available at www.tgrbio.com.

ELISAONE™ phospho-STAT5 assay performance

Using the ELISAONE phospho-STAT5 all-in-one-well assay protocol, the amount of THP-1 cells required to detect STAT5 in GM-CSF-treated cells was examined. THP-1 cells were resuspended at 5×10^6 cells/mL in HBSS/5% FBS, and incubated at 37°C for 1 h. Cells (20 μ L) were then seeded at various densities in a rinsed ELISAONE assay plate, and stimulated with 20 μ L of 20 ng/mL GM-CSF for 10 mins. Cells were lysed using freshly prepared Lysis Mix Concentrate, with shaking for 10 min, and analyzed for phospho-STAT5 using the ELISAONE protocol.



ELISAONE STAT5 assays are optimized for the rapid detection of STAT5 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 μ L/well freshly prepared Lysis Mix, with shaking for 10 min. Lysates (50 μ L) were transferred to an ELISAONE assay plate and analyzed for either phospho STAT5 using the standard ELISAONE protocol. Briefly, Antibody Mix specific for phospho-STAT5 (50 μ L/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-STAT5 (p-Tyr694/699)	24 assays	ERS006
	96 assays	EKT006

* 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- κ B Signaling:

NF- κ B p65, I- κ B α

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.