

## Product datasheet

# STAT3 (pY705 + Total) ELISA Kit ab176666

SimpleStep ELISA<sup>®</sup>

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### Overview

**Product name** STAT3 (pY705 + Total) ELISA Kit

**Detection method** Colorimetric

**Precision**

Intra-assay

Sample	n	Mean	SD	CV%
STAT3(pY705)	6			3.4%
STAT3(Total)	6			2.9%

Inter-assay

Sample	n	Mean	SD	CV%
STAT3(pY705)	3			5.4%
STAT3(Total)	3			7.4%

**Sample type** Cell Lysate, Tissue Homogenate

**Assay type** Semi-quantitative

**Assay time** 1h 30m

**Assay duration** One step assay

**Species reactivity** **Reacts with:** Human

**Predicted to work with:** Mouse, Rat

**Product overview**

Abcam's STAT3 (pY705) and STAT3 (Total) *in vitro* SimpleStep ELISA™ (Enzyme-Linked Immunosorbent Assay) kit is designed for the semi-quantitative measurement of STAT3 (pY705) and Total STAT3 protein in Human cells.

The SimpleStep ELISA™ employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to

the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

**As of October 2019, this kit was reformulated with new antibodies to maintain continued long term supply.**

**Notes** **Estimated sensitivity:** Phospho-STAT3 (Tyr705): 30 µg/mL (tested with HeLa), Total STAT3: 15 µg/mL (tested with A431)  
**Range:** Phospho-STAT3 (Tyr705): 20-2000 µg/mL, Total STAT3: 30-3000 µg/mL

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**Platform** Microplate

**Properties**

**Storage instructions** Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
10X Wash Buffer PT	1 x 15ml
50X Cell Extraction Enhancer Solution	1 x 1ml
5X Cell Extraction Buffer PTR	1 x 12ml
Lyophilized STAT3 Control Lysate	1 vial
Plate Seal	1 unit
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit
STAT3 (pY705) Capture Antibody	1 x 1.5ml
STAT3 (pY705) Detector Antibody	1 x 1.5ml
STAT3 (Total) Capture Antibody	1 x 1.5ml
STAT3 (Total) Detector Antibody	1 x 1.5ml
Stop Solution	1 x 12ml
TMB Substrate	1 x 12ml

**Function** Signal transducer and transcription activator that mediates cellular responses to interleukins,

KITLG/SCF, LEP and other growth factors. Once activated, recruits coactivators, such as NCOA1 or MED1, to the promoter region of the target gene (PubMed:17344214). May mediate cellular responses to activated FGFR1, FGFR2, FGFR3 and FGFR4. Binds to the interleukin-6 (IL-6)-responsive elements identified in the promoters of various acute-phase protein genes. Activated by IL31 through IL31RA. Involved in cell cycle regulation by inducing the expression of key genes for the progression from G1 to S phase, such as CCND1 (PubMed:17344214). Mediates the effects of LEP on melanocortin production, body energy homeostasis and lactation (By similarity). May play an apoptotic role by transactivating BIRC5 expression under LEP activation (PubMed:18242580). Cytoplasmic STAT3 represses macroautophagy by inhibiting EIF2AK2/PKR activity.

**Tissue specificity**

Heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas.

**Involvement in disease**

Hyperimmunoglobulin E recurrent infection syndrome, autosomal dominant Autoimmune disease, multisystem, infantile-onset

**Sequence similarities**

Belongs to the transcription factor STAT family.  
Contains 1 SH2 domain.

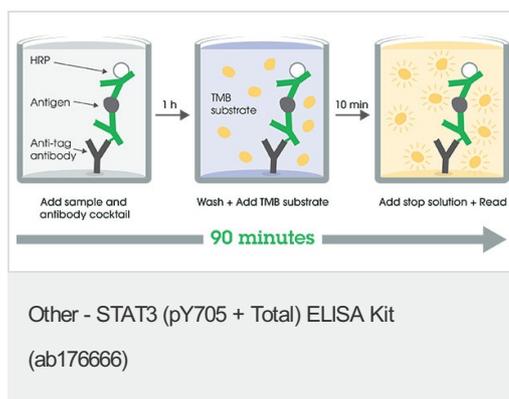
**Post-translational modifications**

Tyrosine phosphorylated upon stimulation with EGF. Tyrosine phosphorylated in response to constitutively activated FGFR1, FGFR2, FGFR3 and FGFR4 (By similarity). Activated through tyrosine phosphorylation by BMX. Tyrosine phosphorylated in response to IL6, IL11, LIF, CNTF, KITLG/SCF, CSF1, EGF, PDGF, IFN-alpha, LEP and OSM. Activated KIT promotes phosphorylation on tyrosine residues and subsequent translocation to the nucleus. Phosphorylated on serine upon DNA damage, probably by ATM or ATR. Serine phosphorylation is important for the formation of stable DNA-binding STAT3 homodimers and maximal transcriptional activity. ARL2BP may participate in keeping the phosphorylated state of STAT3 within the nucleus. Upon LPS challenge, phosphorylated within the nucleus by IRAK1. Upon erythropoietin treatment, phosphorylated on Ser-727 by RPS6KA5. Phosphorylation at Tyr-705 by PTK6 or FER leads to an increase of its transcriptional activity. Dephosphorylation on tyrosine residues by PTPN2 negatively regulates IL6/interleukin-6 signaling.

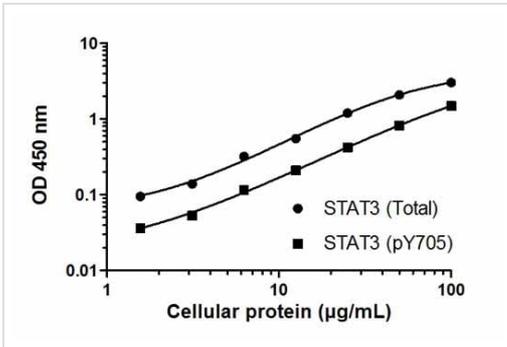
**Cellular localization**

Cytoplasm. Nucleus. Shuttles between the nucleus and the cytoplasm. Translocated into the nucleus upon tyrosine phosphorylation and dimerization, in response to signaling by activated FGFR1, FGFR2, FGFR3 or FGFR4. Constitutive nuclear presence is independent of tyrosine phosphorylation. Predominantly present in the cytoplasm without stimuli. Upon leukemia inhibitory factor (LIF) stimulation, accumulates in the nucleus. The complex composed of BART and ARL2 plays an important role in the nuclear translocation and retention of STAT3. Identified in a complex with LYN and PAG1.

**Images**

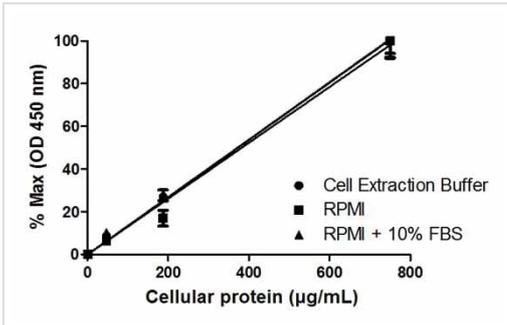


SimpleStep ELISA technology allows the formation of the antibody-antigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.



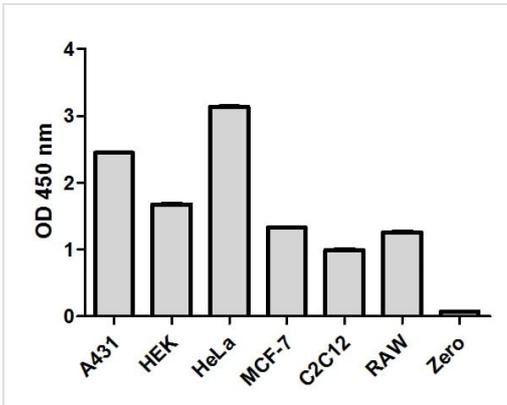
Typical cell lysate dilution series

Example of a typical STAT3 (pY705) and STAT3 (Total) cell lysate dilution series. Background-subtracted data values (mean +/- SD) are graphed.



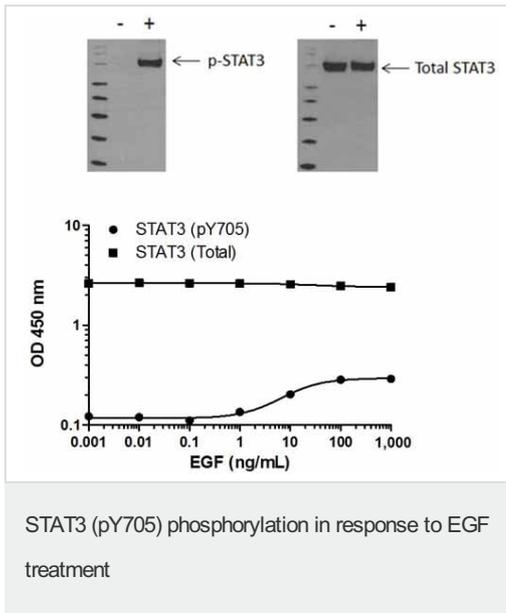
Linearity of dilution

Linearity of dilution in representative sample matrices. Cellular lysates were prepared at 3 concentrations in common media containing 1X Cell Extraction Buffer PTR. Data from duplicate measurements of STAT3 (pY705) are normalized and plotted.



Comparison of STAT3 expression in different cell lines

Cell line analysis for Total STAT3 from 200 µg/mL preparations of cell extracts. Data from triplicate measurements (mean +/- SD) are plotted and compared to 1X Cell Extraction Buffer PTR (zero).



Induction of STAT3 (pY705) phosphorylation in A431 cells in response to EGF treatment. A431 cells were cultured in 96-well tissue culture plates and treated (15 min) with a dose-range of EGF before cell lysis. Data from quadruplicate measurements of STAT3 (pY705) are plotted and compared against total STAT3 protein levels. Comparative STAT3 (pY705) and STAT3 (Total) data also shown by Western Blot.

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