

## Product datasheet

# STAT 5 A/B (pY694/699 + Total) ELISA Kit ab205715

SimpleStep ELISA<sup>®</sup>

[1 References](#) [4 Images](#)

### Overview

**Product name** STAT 5 A/B (pY694/699 + Total) ELISA Kit

**Detection method** Colorimetric

**Precision**

Intra-assay

| Sample    | n | Mean | SD | CV%  |
|-----------|---|------|----|------|
| pY694/699 | 6 |      |    | 2.8% |
| Total     | 6 |      |    | 3.9% |

Inter-assay

| Sample    | n | Mean | SD | CV%  |
|-----------|---|------|----|------|
| pY694/699 | 3 |      |    | 4.3% |
| Total     | 3 |      |    | 2.1% |

**Sample type** Cell culture extracts, Cell Lysate

**Assay type** Semi-quantitative

**Sensitivity** 5 µg/ml

**Assay time** 1h 30m

**Assay duration** One step assay

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

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

**Product overview**

Abcam's STAT5 A/B (pY694/699) and STAT5 A/B (Total) in vitro SimpleStep ELISA<sup>®</sup> (Enzyme-Linked Immunosorbent Assay) kit ([ab204715](#)) is designed for the semi-quantitative measurement of STAT5 A/B (pY694/699) and Total STAT5 A/B protein in Human cells.

The SimpleStep ELISA<sup>®</sup> 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

An alternative fluorescent substrate, ADHP, can be used with this assay. A microplate reader capable of measuring fluorescence is required if using this product.

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

Abcam's STAT5 A/B (pY694/699) and STAT5 A/B (Total) SimpleStep ELISA® kit ([ab204715](#)) has been re-developed with new detection antibody. From February 18, 2016 the kit will be delivered with a new detection total STAT5 antibody. This change was necessary because of a decrease in the performance of the polyclonal STAT5 antibody that was previously used for capture in recent production lots. We have identified a new antibody to use in the SimpleStep ELISA platform that provides higher performance.

**Platform** Pre-coated microplate (12 x 8 well strips)

## Properties

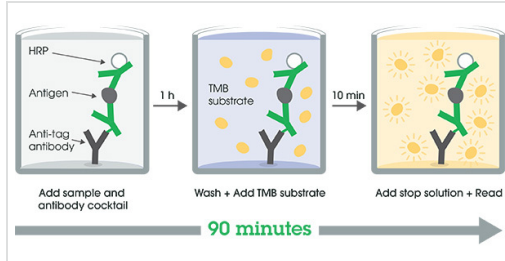
**Storage instructions** Store at +4°C. Please refer to protocols.

| 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 ( <a href="#">ab193970</a> )    | 1 x 10ml     |
| Lyophilized STAT5 A/B Control Lysate                          | 1 vial       |
| Plate Seal  | 1 unit       |
| SimpleStep Pre-Coated 96 Well Microplate (12 x 8 well strips) | 1 unit       |
| STAT5 A/B (pY694/699) Capture Antibody                        | 1 x 1.5ml    |
| STAT5 A/B (pY694/699) Detector Antibody                       | 1 x 1.5ml    |
| STAT5 A/B (Total) Capture Antibody                            | 1 x 1.5ml    |
| STAT5 A/B (Total) Detector Antibody                           | 1 x 1.5ml    |
| Stop Solution   | 1 x 12ml     |
| TMB Substrate   | 1 x 12ml     |

## Cellular localization

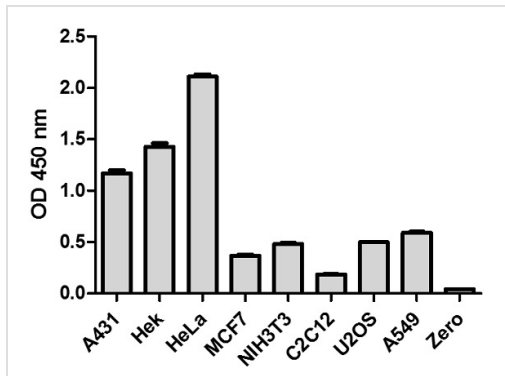
STAT5a + STAT5b: Cytoplasm. Nucleus. Translocated into the nucleus in response to phosphorylation. STAT5 (Total): Cytoplasm. Nucleus. Translocated into the nucleus in response to phosphorylation.

## Images



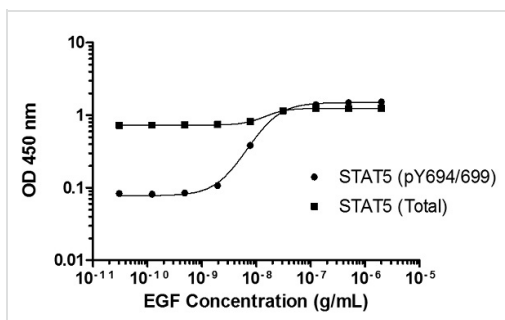
SimpleStep ELISA technology allows the formation of the antibody-antigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.

Other - STAT 5 A/B (pY694/699 + Total) ELISA Kit (ab205715)



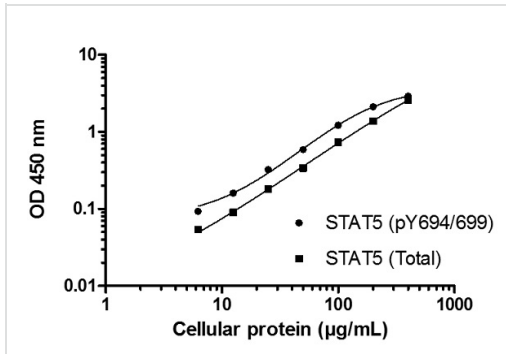
Cell line analysis for Total STAT5 A/B from 100 µg/mL preparations of cell extracts. Data from triplicate measurements (mean +/- SD) are plotted and compared to 1X Cell Extraction Buffer PTR (zero).

Cell line analysis for Total STAT5 A/B from 100 µg/mL preparations of cell extracts



Induction of STAT5 A/B (pY694/699) 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 STAT5 A/B (pY694/699) are plotted and compared against Total STAT5 A/B levels.

Induction of STAT5 A/B (pY694/699) phosphorylation in A431 cells in response to EGF treatment.



Example of a typical STAT5 A/B (pY694/699) and STAT5 A/B (Total) cell lysate dilution series. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

Example of a typical STAT5 A/B (pY694/699) and STAT5 A/B (Total) cell lysate dilution series

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