# STAT3 [pY705] ELISA Kit

**Catalog nos.** KH00481

**Pub. No**. MAN0003940 **Rev** 3.0

## Description

The STAT3 [pY705] ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of STAT3 phosphorylated at tyrosine residue 705 in lysates of human, mouse, and rat cells and tissues.

## Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2°C to 8°C.

| Components | Cat. no. KH00481<br>96 tests |
|---|---|
| STAT3 [pY705] Antibody Coated Wells. 96 well plate. | 1 plate |
| STAT3 [pY705] Detection Antibody. Contains 0.1% sodium azide, blue dye*. | 11 mL |
| STAT3 [pY705] Standard. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume. | 1 vials |
| Wash Buffer Concentrate (25X). | 100 mL |
| Standard Diluent Buffer. Contains 0.1% sodium azide, red dye*. | 25 mL |
| Anti-Rabbit IgG HRP (100X). Contains 3.3 mM thymol. | 0.125 mL |
| HRP Diluent. Contains 3.3 mM thymol, yellow dye*. | 25 mL |
| Stabilized Chromogen, Tetramethylbenzidine (TMB). | 25 mL |
| Stop Solution. | 25 mL |
| Adhesive Plate Covers. | 3 |

* To avoid pipetting mistakes, colored Standard Diluent Buffer, Detection Antibody, and HRP Diluent are provided to monitor the addition of solution to each well. Dyes do not interfere with test results.

**CAUTION!** This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

## Materials required but not provided

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

## General Guidelines

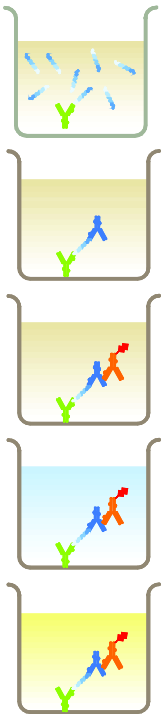
- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com/techresources** for details prior to starting the procedure.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

## ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 4 hours.**

**IMPORTANT!** Perform a standard curve with each assay.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use.



### Bind antigen

1. Add 100 µL Standard Diluent Buffer to zero standard wells. Wells for chromogen blank should be left empty.
2. Add 100 µL of standards and diluted samples (see Dilute samples) to the appropriate wells.
3. Cover the plate with the plate cover and incubate for 2 hours at room temperature.
4. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

### Add detector antibody

5. Add 100 µL STAT3 [pY705] Detection Antibody solution into each well except the chromogen blanks.
6. Cover the plate with plate cover and incubate for 1 hour at room temperature.
7. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

### Add Anti-Rabbit IgG HRP

8. Add 100 µL Anti-Rabbit IgG HRP solution (see page 2) into each well except the chromogen blanks.
9. Cover the plate with the plate cover and incubate for 30 minutes at room temperature.
10. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

### Add chromogen

11. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
12. Incubate for 30 minutes at room temperature **in the dark**.
    **Note:** TMB should not touch aluminum foil or other metals.

### Add stop solution

13. Add 100 µL of Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.


## Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

**Note:** Dilute samples producing signals greater than that of the highest standard in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

## Performance characteristics

### Standard curve (example)

The following data were obtained for the various standards over the range of 0-100 Units/mL STAT3 [pY705].

| Standard STAT3 [pY705] (Units/mL) | Optical Density (450 nm) |
|---|---|
| 100 | 2.79 |
| 50 | 1.86 |
| 25 | 1.11 |
| 12.5 | 0.62 |
| 6.25 | 0.39 |
| 3.13 | 0.29 |
| 1.57 | 0.24 |
| 0 | 0.20 |

### Recovery

To evaluate recovery, Cell Extraction Buffer was diluted 1:10 with Standard Diluent Buffer to bring the SDS concentration to <0.01%. STAT3 [pY705] Standard was spiked into this. The average recovery was 93%.

## Performance characteristics, continued

### Intra-assay precision

Samples of known STAT3 [pY705] concentration were assayed in replicates of 16 to determine precision within an assay.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| Mean (pg/mL) | 35.0 | 16.0 | 8.3 |
| SD | 1.2 | 0.3 | 0.5 |
| %CV | 3.5 | 2.1 | 6.1 |

SD = Standard Deviation; CV = Coefficient of Variation

### Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| Mean (pg/mL) | 35.5 | 16.1 | 7.9 |
| SD | 1.3 | 0.4 | 0.6 |
| %CV | 3.6 | 2.6 | 7.2 |

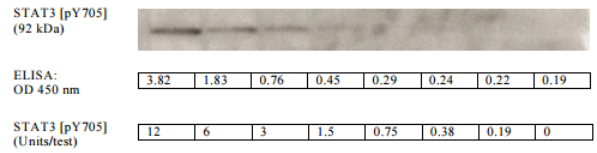
SD = Standard Deviation: CV = Coefficient of Variation

### Sensitivity

The minimum detectable dose of STAT3 [pY705] is <0.9 Units/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times, and calculating the corresponding concentration.

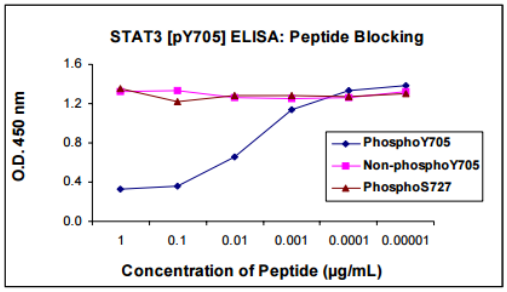
The sensitivity of this ELISA was compared to Western blotting using known quantities of STAT3 [pY705]. The data presented below show that the sensitivity of the ELISA is approximately 5x greater than that of Western blotting. The bands shown in the Western blot data were developed using rabbit anti-STAT3 [pY705] and an alkaline phosphatase conjugated anti-rabbit IgG followed by chemiluminescent substrate and autoradiography.

**Detection of STAT3 [pY705] by ELISA vs Western Blot:**




### Specificity

The specificity of this assay for STAT3 phosphorylated at tyrosine 705 was confirmed by peptide competition. The data presented below show that the phospho-peptide containing the phosphorylated tyrosine 705 blocked the ELISA signal. The same STAT3 sequences without phosphate groups did not block the ELISA signal.



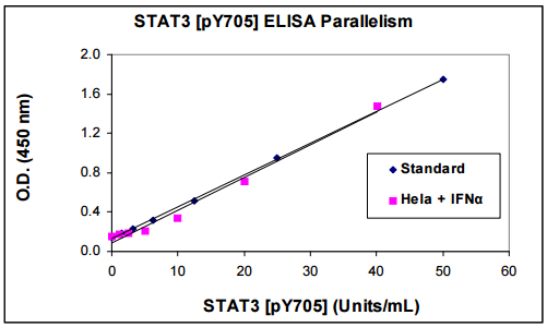

### Linearity of dilution

HeLa cells were grown in tissue culture medium containing 10% fetal bovine serum, treated with 50 ng/mL IFN-α for 15 minutes and lysed with Cell Extraction Buffer. This lysate was diluted in Standard Diluent Buffer over the range of the assay and measured for STAT3 [pY705]. Linear regression analysis of sample values versus the expected concentration yielded a correlation coefficient of 0.99.

| Dilution | Cell Lysate | | |
|---|---|---|---|
| | Measured (Units/mL) | Expected (Units/mL) | % Expected |
| Neat | 24.2 | 24.2 | 100 |
| 1/2 | 12.5 | 12.1 | 103 |
| 1/4 | 6.6 | 6.1 | 109 |
| 1/8 | 3.2 | 3.0 | 107 |
| 1/16 | 1.78 | 1.5 | 117 |

### Parallelism

Natural STAT3 [pY705] from IFN-α-treated HeLa cell lysate was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the STAT3 [pY705] standard curve. Parallelism demonstrated by the figure below indicated that the standard accurately reflects STAT3 [pY705] content in samples.




## Important licensing information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

## Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

## Product label explanation of symbols and warnings

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| REF | Catalog Number | LOT | Batch code | | Temperature limitation | | Use by | | Manufacturer | | Consult instructions for use | | Caution, consult accompanying documents |



For support visit thermofisher.com/support or contact techsupport@lifetech.com.

thermofisher.com

26 January 2016

## Prepare 1X Wash Buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
3. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

## Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

## Dilute samples

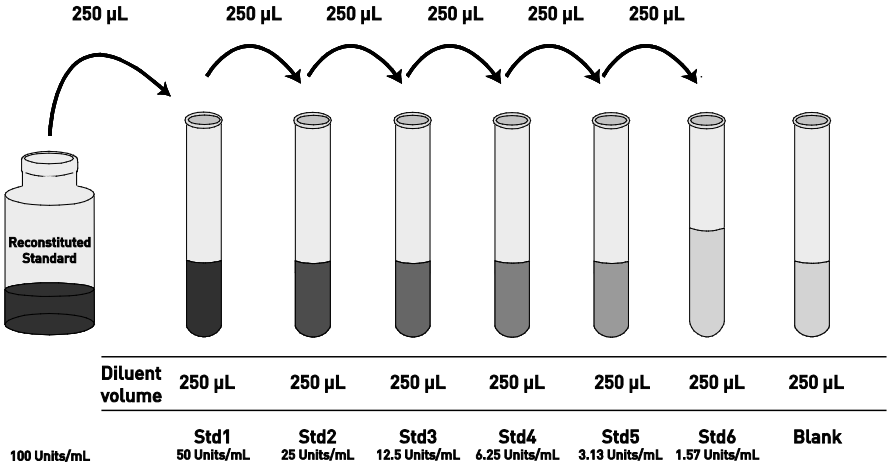
- Dilute samples prepared in Cell Extraction Buffer 10-fold or greater in Standard Diluent Buffer.
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

## Dilute standards

**Note:** The STAT3 [pY705] Standard is prepared using purified, recombinant, phosphorylated STAT3 protein. One Unit of standard is equivalent to the amount of STAT3 [pY705] derived from 5 pg of STAT3 that was phosphorylated by activated JAK.

**Note:** Use glass or plastic tubes for diluting standards.

1. Reconstitute STAT3 [pY705] Standard to 100 Units/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 100 Units/mL STAT3 [pY705].
   **Use the standard within 1 hour of reconstitution**.
2. Add 250 µL Reconstituted Standard to one tube containing 250 µL Standard Diluent Buffer and label as 50 Units/mL STAT3 [pY705].
3. Add 250 µL Standard Diluent Buffer to each of 5 tubes labeled as follows: 25, 12.5, 6.25, 3.13, and 1.57 Units/mL STAT3 [pY705].
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. Discard remaining reconstituted standard or freeze in aliquots at -80°C. Standard can be frozen and thawed one time only without loss of activity. Return Standard Diluent Buffer to the refrigerator.




## Prepare Anti-Rabbit IgG HRP solution

**Note:** Prepare Anti-Rabbit IgG HRP within 15 minutes of usage.

The Anti-Rabbit IgG HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

1. For each 8-well strip used in the assay, pipet 10 µL Anti-Rabbit IgG HRP (100X) solution, wipe the pipette tip with a clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
2. Return the unused Anti-Rabbit IgG HRP (100X) solution to the refrigerator.