



# STAT1, 3, 5a/b Phospho 3-Plex Kit INFORMATION SHEET

Catalog #: LHO0005 Description: STAT1, 3, 5a/b Phospho 3-Plex Kit Lot:\* A10856

\*Note: A letter at the end of the lot number signifies an additional packaging of this same lot.

## Intended Use

This reagent set comprises the analyte specific components for the measurement of the phosphorylated proteins STAT1 [pY701], STAT3 [pY705], and STAT5a/b [pY694/699] ([pY694] for STAT5a and [pY699] for STAT5b) in cell lysates and tissue homogenates. These reagents are intended for use in the **Luminex® 100™ or 200™ System** only.

**Before attempting to multiplex this kit with any other kits, please contact Technical Support.**

**This kit is configured for research use only and is not to be used in diagnostic procedures.**

## Reagents Provided

### 1. Antibody Bead Concentrate (10x):

Catalog #: LM209 Description: STAT1, 3, 5a/b Phospho 3-Plex Beads Lot: A10854 Size: 0.25 mL-100 tests

Analyte:	Bead Region:
STAT1 [pY701]	44
STAT3 [pY705]	30
STAT5a/b [pY694/699]	28

**Form:** 0.25 mL 10x bead concentrate solution in storage buffer. Contains 7.5 mM sodium azide as a preservative.

**Storage:** **Light sensitive material.** Store at 2 to 8°C, in the dark, until the expiration date indicated on the kit.

### 2. Detection Antibody Concentrate (10x):

Catalog #: DN209 Description: STAT1, 3, 5a/b Phospho 3-Plex Detector Lot: A10855 Size: 0.50 mL-100 tests

**Form:** 0.5 mL of a 10x stock of Detection Antibody Concentrate. Contains 15 mM sodium azide as preservative. Concentration of antibody is matched to this lot of beads. Do not mix lots of Coated Beads and Detection Antibody.

**Storage:** Store at 2 to 8°C until the expiration date indicated on the kit.

### 3. Standard (2 vials):

Catalog #: SM209 Description: STAT1, 3, 5a/b Phospho 3-Plex Standard Lot: A10823 Size: 2 vials

**Form:** This standard (lyophilized recombinant protein) is designated in Units/mL. The protein in this standard has been calibrated with its respective Invitrogen ELISA kits when available. Detailed information on calibration is provided below.

**Storage:** Store at 2 to 8°C. Use within 1 hour after reconstitution. The remaining reconstituted standard should be discarded or frozen in aliquots at -80°C for further use. The standard can be frozen and thawed one time without loss of immunoreactivity.

### Concentration of Reconstituted Standards\*\*:

STAT1 [pY701] (100 Units/mL)
STAT3 [pY705] (180 Units/mL)
STAT5a/b [pY694/699] (200 Units/mL)

**\*\*Important note: The concentrations of reconstituted standards are lot-specific. Please verify all concentration values entered in data analysis software.**

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**Reconstitution:** Reconstitute in **1.5 mL Assay Diluent**.

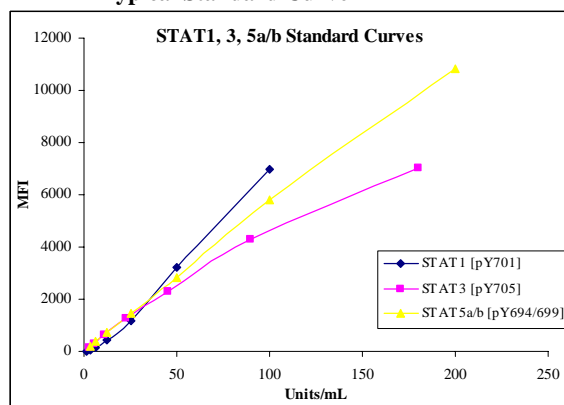
**Recommended Starting Concentration for Standard Curve:** Upon reconstitution, the starting concentration of standards is the value cited above. Make 2-fold serial dilutions in *Assay Diluent*. See the Product Insert included in the kit for further information.

**Calibration:** STAT1 [pY701] and STAT3 [pY705] were calibrated to their respective Invitrogen ELISA kits, KHO0271 (STAT1 [pY701]) and KHO0481 (STAT3 [pY705]). Subsequent lots of standard will be normalized to this lot of material to allow consistency of quantitation.

**Species Reactivity:** This reagent set recognizes human, mouse, and rat proteins. Other species have not been tested.

**Performance Characteristics**

**Typical Standard Curve**



Note: This is a representative standard curve. Absolute MFI values will vary.

Protein	STAT1 [pY701]	STAT3 [pY705]	STAT5a/b [pY694/699]
Sensitivity (units/mL) <sup>1</sup>	1.3	0.15	0.5
Recovery (10% FNN0011) <sup>2</sup>	90%	110%	109%
Recovery (10% FNN0021) <sup>2</sup>	99%	109%	108%
Intra-assay variation (n=16)	6.6%	4.4%	3.2%
Inter-assay variation (n=32)	8.1%	5.6%	3.8%
Invitrogen ELISA (Catalog #)	KHO0271	KHO0481	N/A

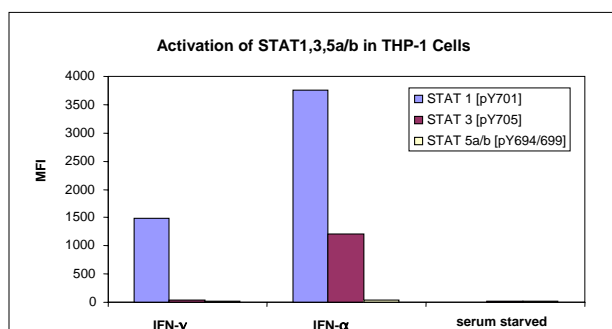
<sup>1</sup>**Analytical Sensitivity:** The analytical sensitivity was determined by adding two standard deviations to the median fluorescence intensity obtained when the zero standard was assayed 32 times.

<sup>2</sup>**Recovery:** To evaluate recovery, analytes were spiked at 3 different concentrations into 10% Cell Extraction Buffer (Cat. # FNN0011) and 10% NP40 Cell Lysis Buffer (Cat. # FNN0021).

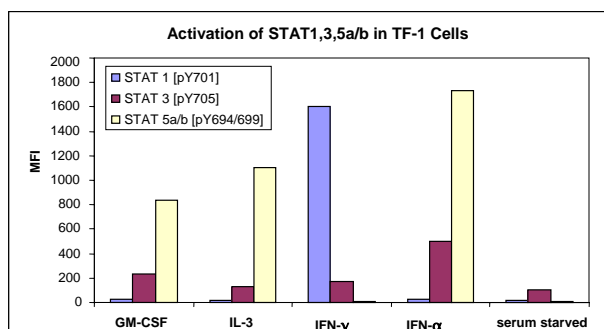
**Sample Preparation:** This kit has been validated with cell lysates prepared in Cell Extraction Buffer (Cat. # FNN0011) and NP40 Cell Lysis Buffer (Cat. # FNN0021). Optimization of cell stimulation and cell lysis procedures may be required for each specific application. Refer to Product Insert for detailed instructions.

**Example Data:** To evaluate the performance of this kit, cell lines were treated with various stimulants to activate the STAT proteins. In figure A, THP-1 cells were serum starved overnight, treated with either 100 ng/mL IFN- $\gamma$  or 10 ng/mL IFN- $\alpha$  for 15 minutes, or left untreated (Figure A). TF-1 cells were treated with GM-CSF (100 ng/mL), IL-3 (100 ng/mL), IFN- $\gamma$  (100 ng/mL), IFN- $\alpha$  (10 ng/mL) for 15 minutes, or left untreated (Figure B). Cell lysates were prepared and analyzed with the assay. In figure B, due to the relatively high levels of STAT5a/b [pY694/699] in the samples, signals from 50  $\mu$ g/mL of the cell lysates were presented in the figure, while MFIs (median fluorescence intensity) for STAT1 [pY701] and STAT3 [pY705] were from 400  $\mu$ g/mL of the cell lysates.

**A**



**B**



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