

Measuring proteins in JAK-STAT signaling

QUANTITATE SITE-SPECIFIC PROTEIN PHOSPHORYLATION USING PHOSPHOELISA™ ASSAYS.

The JAK-STAT signaling pathway plays an important role in regulating cell proliferation, differentiation, and apoptosis,^{1,2} making identification of inhibitors of the JAK-STAT protein family a focus in oncology and immune suppression research. The phosphorylation status of the proteins involved in the JAK-STAT pathway largely determines the direction of downstream activity. Now you can accurately quantitate phosphorylated protein levels in minimal sample amounts using BioSource™ phosphoELISA™ Kits. These easy-to-perform assays provide highly specific results in as little as four hours.

Following phosphorylation in the JAK-STAT pathway

Upon binding of a variety of cytokines and growth factors to the appropriate receptor, JAK kinases are recruited and activated. JAKs then phosphorylate the receptor’s cytoplasmic domain, causing recruitment of STATs, which are in turn phosphorylated, dimerized, and translocated into the nucleus. Once in the nucleus, STAT family members (STAT1, 2, 3, 4, 5a, 5b, and 6) control transcription of specific

genes in response to stimulation. Although this basic mechanism of the JAK-STAT pathway is known, very little is known about the details of the recruitment of particular JAK or STAT proteins. For example, cytokine receptors demonstrate preferential use of either a single JAK or a JAK combination, as shown by genetic studies. STATs, on the other hand, have been shown to exhibit either cell type-specific induction of transcription or stereotypic transcription, regardless of cell type. Further studies are required to examine and clarify these restrictions. Since phosphorylation appears to contribute to the activity of these proteins, measuring these levels using the BioSource™ phosphoELISA™ Kits will hopefully help uncover some answers.

Measuring specific phosphorylation

BioSource™ phosphoELISA™ Kits from Invitrogen are now available to specifically measure total and site-specific STAT protein phosphorylation. These simple, unbiased assays enable you to determine STAT protein levels with high specificity and sensitivity in only

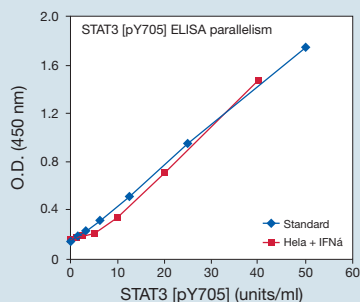


Figure 1—phosphoELISA™ Kit standards parallel natural samples. Recombinant standards are tested against cell lysates to ensure correct measurement values of natural samples. Note that the standard is parallel to the natural sample used.

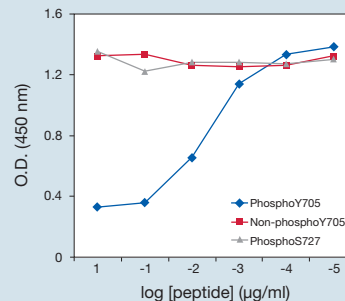


Figure 2—High specificity of the phosphoELISA™ Kit—peptide competition. Peptide blocking is performed on each kit to confirm specificity of the phosphorylation site. The phosphorylated tyrosine 705 blocked the ELISA signal, but not the nonphosphorylated peptide sequence or another phosphopeptide.

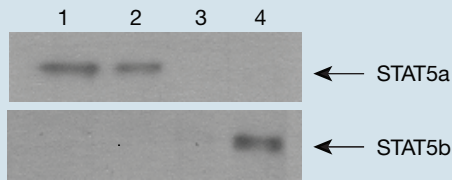


Figure 3—High specificity of the phosphoELISA™ Kit—no cross-reactivity. HEL cell lysates were incubated with the capture antibody used in the STAT5a [pY694] ELISA (lane 2). An antibody specific for STAT5a and STAT5b was used as a positive control (lanes 1 and 4). IgG beads were used as a negative control (lane 3). The capture antibody recognizes the a isoform of STAT5 but not the b isoform. Thus, the STAT5a [pY694] ELISA does not cross-react with the STAT5b protein.

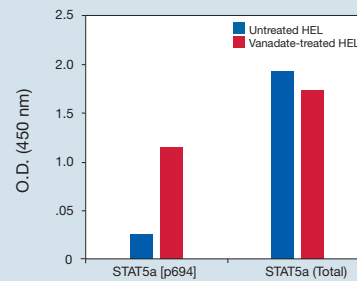


Figure 4—Ensure correct activation patterns. Cell extracts were prepared and analyzed with the STAT5a [pY694] ELISA and STAT5a (Total) ELISA kits. Phosphorylation of STAT5a is increased in sodium vanadate-treated HEL cells, whereas the total level of STAT5a remains relatively constant in treated vs. untreated control, demonstrating the utility of the Total ELISA kits as controls.

four hours. All phosphoELISA™ Kits are rigorously tested to ensure excellent quality. Validation studies include experiments to verify parallelism between calibrated standards and natural samples (Figure 1), peptide competition and cross-reactivity tests for specificity (Figures 2 and 3), and stimulation experiments to ensure correct activation patterns (Figure 4). Specifications include excellent precision (<10% CV), recovery (85–108%), and sensitivity (at least 2x more sensitive than western blots). phosphoELISA™ Kits can be combined with western blot images of specific protein bands to ensure confidence in your results (Figure 5).

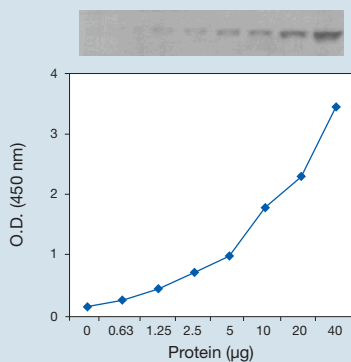


Figure 5—Confirm western data. In parallel, cell extracts were prepared and analyzed with western blots and phosphoELISA™ kits for STAT5 [pY694]. The O.D. reading corresponds to the intensity of the bands in the western blot.

Completely optimized phosphoELISA™ Kits are supplied ready to use with all the necessary reagents, including recombinant standards to be used as positive controls and for quantitative results. In addition, the flexible 96-stripwell plate format allows you to run as many samples, or as few, as you need. For complete information on the Invitrogen™ tools available for studying the JAK-STAT pathway, visit www.invitrogen.com/pelisa. ■

References

- Ihle, J.N. (1996) *Cell* 84(3):331–334.
- Murray, P.J. (2007) *J Immunol* 178:2623–2629.

Product	Species	Quantity	Cat. no.
Antibodies			
JAK/STAT Sampler	Hu	2 blots	44-429G
JAK1	Hu	100 µg	AHO1512
JAK2 (mAb)	Hu, Ms, Rt	100 µg	AHO1352
STAT3	Hu, Ms, Rt	100 µg	AHO1252
STAT4 (mAb)	Hu, Ms, Rt	100 µg	AHO1342
STAT5 (mAb)	Hu, Ms	100 µg	AHO1462
STAT6 (mAb)	Hu, Ms, Rt	100 µg	AHO1452
phosphoELISA™ kits			
STAT1 [pY701]	Hu	96 tests	KHO0271
STAT1 (Total)	Hu	96 tests	KHO0261
STAT3 [pY705]	Hu, Ms, Rt	96 tests	KHO0481
STAT3 (Total)	Hu, Ms, Rt	96 tests	KHO0471
STAT5a [pY694]	Hu	96 tests	KHO0761
STAT5a (Total)	Hu	96 tests	KHO0751
STAT6 [pY641]	Hu	96 tests	KHO0801
Luminex® assays			
STAT1 (Total)	Hu	96 tests	LHO0261
STAT1 [pY701]	Hu	96 tests	LHO0271
STAT3 [pY705]	Hu, Ms, Rt	96 tests	LHO0481