# Keep your discovery on track



## **ELISA** kits for signaling protein targets

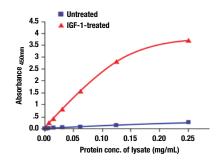
- Single-wash assay protocol reduces hands-on time and assay complexity
- In-house CST testing processes make sure results are reproducible across lots and users
- Validated for specificity and accuracy across biological sample types
- Integrated controls ensure assay performance

FastScan™ ELISA kits let you move through research and discovery phases faster. We've identified optimal antibody pairs and done the assay development and validation up front to make sure your results are accurate across the lifetime of your discovery project — from target validation through preclinical phases.

Our ready-to-go kits use a simple protocol that easily integrates into your current processes and budget, streamlines assay transfer, and lets you rapidly detect targets. Kits are available for both phosphorylation-specific and total protein options and come with integrated controls so your day-to-day results stay on track.

FastScan ELISA kits cover disease targets in cell biology, epigenetics, immuno-oncology, and neuroscience and include a wide selection of assays to measure key cellular signaling proteins.

#### Easy-to-use, sensitive ELISA detection for a range of disease targets



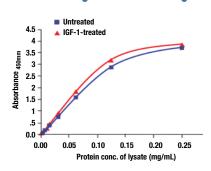
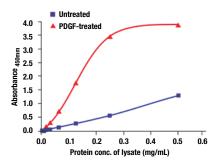


Figure 1. Detection of endogenous levels of Phospho-Akt1 (Ser473) and total Akt1. Treatment of MCF7 cells with IGF-1 stimulates phosphorylation of Akt1 at Ser473 but does not affect the level of total Akt1. The relationship between lysate protein concentration from untreated and IGF-1-treated MCF7 cells (100 ng/ml IGF-1 #8917 for 5 minutes at 37°C) and absorbance using the FastScan™ Phospho-Akt1 (Ser473) ELISA Kit #80895 (top Ieft panel) and the FastScan™ Total Akt1 ELISA Kit #15942 (top right panel) is shown.



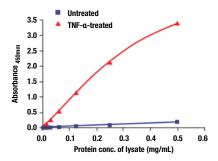
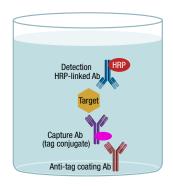


Figure 2 (Left). Detection of endogenous levels of Phospho-CREB (Ser133). Treatment of NIH/3T3 cells with PDGF stimulates phosphorylation of CREB at Ser133. The relationship between lysate protein concentration from untreated and PDGF-treated NIH/3T3 cells (100 ng/ml PDGF #8912 for 5 minutes at 37°C, post serum starvation) and absorbance using the FastScan<sup>™</sup> Phospho-CREB (Ser133) ELISA Kit #42054 is shown.

Figure 3 (Right). Detection of Phospho-IκBα (Ser32).
Treatment of HeLa cells with TNF-α stimulates phosphorylation of IκBα at Ser32. The relationship between lysate protein concentration from untreated and TNF-α-treated HeLa cells (TNF-α [10 ng/ml] #8902 for 5 minutes at 37°C, post serum starvation) and absorbance using the FastScan<sup>TM</sup> Phospho-IκBα (Ser32) ELISA Kit #46530 is shown.

## **How FastScan ELISA technology works**

FastScan ELISAs use a less complex, solution-based assay format with fewer overall steps, so time to results are much faster compared to conventional protocols. You'll also get consistent results across biological sample types with sensitivity that's comparable or better than traditional sandwich ELISA.

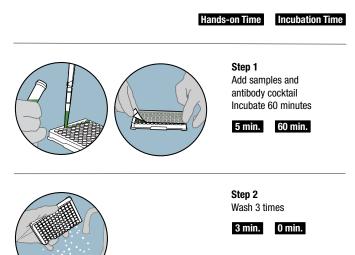


- Sample "target" is incubated with a capture antibody conjugated with a proprietary tag and a second detection antibody linked to horse radish peroxidase (HRP).
- The entire complex is immobilized to a microwell via an anti-tag antibody.
- Wells are washed, followed by enzymatic reaction and readout of target analyte quantity by colorimetric detection.



#### Data in 90 minutes

The simple FastScan ELISA protocol cuts your hands-on time in half and incubation times down by 3X. Sample, along with capture and detection antibodies, are first added to the precoated microwell, then incubated for ~1 hour. The well is then washed 3 times. Next, substrate is added and incubated for 10 to 15 minutes before stop solution is added, then the absorbance signal is measured. The whole process only takes 90 minutes, so you have the results you need quickly.







Cumulative Time 15 min. 75 min.

Total Time 90 min.

#### Results you can trust

CST scientists preselect antibody pairs based on their optimal activity in solution phase. FastScan ELISA kits, along with the recombinant antibodies and reagents that come in each kit, are also developed, produced, and rigorously validated in-house. So you can be absolutely certain of accuracy, lot-to-lot reproducibility, and consistency of results between users and sample types.

#### Assay reproducibility across experiments

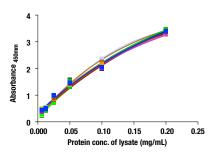


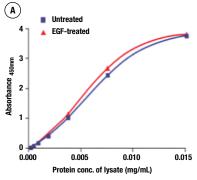
	PLATE A	PLATE B	PLATE C
AVERAGE	1.50	1.42	1.39
STDEV	0.06	0.06	0.02
CV%	4.0%	4.4%	1.7%

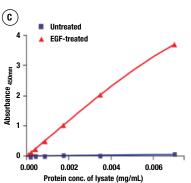
ACROSS PLATE AVERAGE	1.44
ACROSS PLATE STDEV	0.07
ACROSS PLATE CV%	4.8%

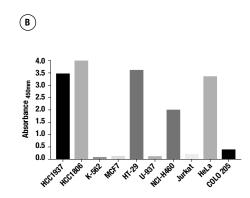
Figure 4. Assay demonstrating typical inter- and intraplate reproducibility. The reproducibility of lysate protein titrations was determined across three plates, with 12 replicate titration curves per plate (36 total titration curves). Shown (left panel) is the relationship between lysate protein concentration from RB-positive Jurkat cells and absorbance using the FastScan<sup>TM</sup> Total Rb ELISA Kit #23595.

The intra-plate CVs (right panel) were found to be <5%, as was the inter-plate CV. Data were calculated off a point from the middle of the titration curve, with 12 data replicates per plate or 36 replicates across three plates. These data are representative of the intra- and inter-plate consistency of the FastScan<sup>TM</sup> ELISA kits.

#### Highly sensitive and specific ELISAs, right out of the box







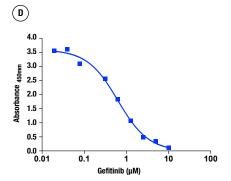


Figure 5. Data demonstrating sensitivity and specificity of FastScan ELISA kits for total and Tyr1068 phosphorylation of EGF receptor.

Treatment of A-431 cells with EGF stimulates phosphorylation of EGF receptor at Tyr1068 but does not affect the level of total EGF receptor protein. The relationship between lysate protein concentration from untreated and EGF-treated A-431 cells (100 ng/ml hEGF #8916 for 5 minutes at 37°C, post-serum starvation) and absorbance using the FastScan™ Total EGF Receptor ELISA Kit #80869 and the FastScan™ Phospho-EGF Receptor (Tyr1068) ELISA Kit #89897 are shown in the left panel. A and C respectively.

Panel B demonstrates the presence or absence of EGF receptor expression in a panel of cell lines using the FastScan<sup>TM</sup> Total EGF Receptor ELISA Kit. This allows for identification of negative and positive EGF receptor cell lines, as well as its relative abundance across EGF receptor-expressing cell lines (+/- EGF receptor cell lines are in agreement with publicly available RNAseq data). Panel D demonstrates the inhibition of EGF-stimulated phosphorylation of EGF receptor at Tyr1068 as detected by the FastScan<sup>TM</sup> Phospho-EGF Receptor (Tyr1068) ELISA, where serum-starved A-431 cells were treated with various concentrations of the potent EGF receptor tyrosine kinase inhibitor gefitinib #4765 (varying doses, 30 minutes) and then stimulated with 100 ng/ml Human Epidermal Growth Factor (hEGF) #8916 for 5 minutes and lysed.

### Kit components

FastScan ELISA kits come with everything you need, including 12 8-well strips for 96 tests.\* Each kit contains:

- FastScan™ ELISA Microwell Strip Plate
- Capture antibody\*\*
- HRP-linked Detection antibody\*\*
- FastScan™ ELISA Capture Antibody Diluent
- FastScan™ ELISA HRP Antibody Diluent
- TMB Substrate
- STOP Solution
- Sealing Tape
- ELISA Wash Buffer (20X)
- FastScan™ Cell Extraction Buffer (5X)
- FastScan<sup>™</sup> Cell Extraction Enhancer Solution (50X)
- FastScan™ ELISA Kit Positive Control\*\*
- \* Bulk packaging available upon request. 384-well format may also be available on a kit-by-kit basis. For more information contact support@cellsignal.com.
- \*\* These components are kit-specific.

## **Recommended companion products**

- Protease/Phosphatase Inhibitor Cocktail (100X), #5872
- FastScan™ ELISA Microwell Strip Plate, #53257
- TMB Substrate, #7004
- STOP Solution, #7002
- ELISA Wash Buffer (20X), #9801
- FastScan™ Cell Extraction Buffer (5X), #69905
- FastScan<sup>™</sup> Cell Extraction Enhancer Solution (50X), #25243





