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# ab207462 Phospho-c-Src (Y418) + Total In-Cell ELISA Kit (Chemiluminescent)

For the quantitative measurement of total and Y418 phosphorylated c-Src in adherent and non-adherent cells.

This product is for research use only and is not intended for diagnostic use.

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# 1. Overview

Phospho-c-Src (Y418) + Total In-Cell ELISA Kit (Chemiluminescent) (ab207462) provides a simple, efficient, cell-based method to monitor proteins activated by phosphorylation. This kit is designed specifically to quantify activated (phosphorylated) c-Src and/or total c-Src. Cells are cultured in 96-well plates and stimulated to induce the pathway of interest. Following stimulation, the cells are rapidly fixed to preserve activation-specific protein modifications. Each well is then incubated with a primary antibody that recognizes either phosphorylated c-Src or total c-Src. Subsequent incubation with secondary HRP-conjugated antibody and chemiluminescent reagent provides an easily quantified chemiluminescent readout. The relative number of cells in each well is then determined using the provided Crystal Violet solution. The 96-well plate format is suitable for high-throughput screening applications.

The Phospho-c-Src (Y418) + Total In-Cell ELISA Kit (Chemiluminescent) (ab207462) contains two 96-well plates and two primary antibodies. The phospho-c-Src (Y418) antibody is specific for phosphorylated c-Src (Y418) and was raised against a synthetic phospho-peptide corresponding to residues surrounding Tyr418 of human c-Src. This antibody recognizes c-Src when phosphorylated at this site and also recognizes Fyn and c-Yes. The total-c-Src antibody recognizes c-Src proteins regardless of the phosphorylation state. This kit can be used to study phosphorylated c-Src relative to cell number or be used to determine c-Src phosphorylation relative to the total c-Src protein found in the cells. Once the phospho-c-Src and total- c-Src signals have been normalized for cell number, a comparison of the ratio of phosphorylated c-Src to total c-Src for each of the cell growth conditions can be made. The provided total-c-Src antibody can be used as a positive control to demonstrate that the cells contain c-Src, the kit reagents are functional and that the protocol is performed correctly. Also, because fixed cells are stable for several weeks, you can prepare many plates simultaneously and then perform the assay when desired.

c-Src (proto-oncogene tyrosine-protein kinase Src) is a 60 kDa tyrosine protein kinase that contains both SH2 and SH3 domains. c-Src is a lipid-modified signaling molecule (myristoylated) involved in cell-cell interactions, cell migration and proliferation through the phosphorylation of many substrates. c-Src is expressed at high levels in differentiated cells

including neurons, platelets and macrophages, and can be activated by phospholipase D, insulin-like growth factor receptor, EGF-receptor, fibroblast growth factor receptor and prolactin receptor binding. c-Src can be phosphorylated on multiple sites to interact with polyoma virus middle T antigen, PYK2, HSP72 and caveolin, and efficient study methods are in high demand.

The Src gene family is represented by at least eight different protein tyrosine kinases that belong to the non-receptor tyrosine kinase family. These protein tyrosine kinases are important regulators of many cellular processes, including cytoskeletal organization, cell-cell contact, DNA synthesis and cellular proliferation. Members of this group of proteins include c-Src, c-Yes, Fyn, Lck, Lyn, Hck, Blk and c-Fgr. The proto-oncogene c-Src is the prototype member of this gene family and is expressed in a broad range of tissues and cells. Elevated c-Src tyrosine kinase activity has been found in many types of human cancers, most notably in breast carcinomas.

The activity of Src family tyrosine kinase (SFK) is determined by an equilibrium between an inactive (phosphorylated) and a primed (dephosphorylated) state regulated by a balance of Csk (C-terminal Src kinase) and its counteracting tyrosine phosphatase(s). Upon cell stimulation, SFKs at a primed state become functionally activated to relay signals into the cells. Thus, perturbation of the equilibrium status of SFK may greatly affect the sensitivity of the cells to extracellular cues.

There are two tyrosine phosphorylation sites with opposing effects in Src. Tyrosine 418 is located in the catalytic SH2 domain and is one of the autophosphorylation sites. Phosphorylation of Tyr418 upregulates the enzyme while phosphorylation of Tyr529 in the C-terminal tail by Csk renders the enzyme less active. SFKs are required for EGF-, PDGF- and CSF-1-stimulated entry to the S-phase of the mitotic cycle in fibroblasts. Similarly, they are activated at mitosis and play a role in cellular division at the G2-M transition phase. Activated c-Src can increase tyrosine phosphorylation of paxillin and is involved in the activation of the Ras/ERK pathway.

## 2. Protocol Summary

Culture cells and treat as desired



Fix cells by replacing the growth medium with 4% (adherent cells) or 8% (non-adherent cells) formaldehyde in PBS for 20 minutes at RT. Wash cells



Quench endogenous peroxide by incubating in Quenching Buffer for 20 minutes at RT. Wash cells



Incubate with Antibody Blocking Buffer for 1 hour at RT. Wash cells



Incubate with primary antibody overnight at +4°C. Wash cells



Incubate with secondary antibody for 1 hour at RT. Wash cells



Add Chemiluminescent Reagent and read chemiluminescence using a luminometer or CCD camera system



Crystal Violet cell staining (Optional)

### 3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.
- Sodium Azide and Formaldehyde are highly toxic chemicals. Appropriate safety precautions (gloves and eye protection) should be used. In addition, formaldehyde is highly toxic by inhalation and should be used only in a ventilated hood.

### 4. Storage and Stability

**Store kit at -20°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

## 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. Materials Supplied

Item	1 x 96 tests	5 x 96 tests	Storage Condition
Phospho-c-Src antibody	1 x 9 $\mu$ L	5 x 9 $\mu$ L	-20°C
Total-c-Src antibody	1 x 9 $\mu$ L	5 x 9 $\mu$ L	-20°C
Anti-rabbit HRP-conjugated IgG	1 x 11 $\mu$ L	5 x 11 $\mu$ L	+4°C
1X Antibody Blocking Buffer	1 x 22 mL	5 x 22 mL	-20°C
1X Antibody Dilution Buffer	1 x 30 mL	5 x 30 mL	-20°C
10X PBS	1 x 120 mL	5 x 120 mL	RT
10% Triton X-100	1 x 10 mL	5 x 10 mL	RT
Crystal Violet Solution	1 x 22 mL	5 x 22 mL	+4°C
Chemiluminescent Reagent	2 x 2 mL	10 x 2 mL	+4°C
Reaction Buffer	2 x 4 mL	10 x 4 mL	+4°C
1% SDS Solution	1 x 22 mL	5 x 22 mL	RT
96-well tissue culture plate	2	10	RT
Plate sealing tape	2	10	RT

## 7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Multi-channel pipettor.
- Multi-channel pipettor reservoirs.
- Rocking platform.
- Parafilm.
- Microplate spectrophotometer capable of reading at 595 nm for Crystal Violet staining.
- Microplate luminometer or CCD camera-coupled imaging system for chemiluminescent detection.
- Fresh 10% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in dH<sub>2</sub>O (3 mL are required).
- 10 µg/mL poly-L-Lysine (if using non-adherent cells).
- 10% Sodium Azide (NaN<sub>3</sub>) in dH<sub>2</sub>O (250 µL are required).
- 37% Formaldehyde (2.5 mL are required for adherent cells; 5.0 mL are required for non-adherent cells).

## 8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

## 9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.
- We provide an excess of buffer components in order to perform one 96-well assay with the phospho-c-Src antibody and one 96-well assay with the total-c-Src antibody.

### 9.1 1X PBS:

Prepare 1X PBS by adding 1 volume of 10X PBS (pH 7.4) to 9 volumes of dH<sub>2</sub>O and mixing thoroughly.

### 9.2 Preparation of Fixing Buffer (4% or 8% Formaldehyde in PBS):

Fixing Buffer is used to fix cells after cell culturing. Prepare by adding formaldehyde to 1X PBS and mixing well. 4% formaldehyde is used with adherent cells; 8% formaldehyde is used with non-adherent cells. The recipe in the Quick Chart for Preparing Buffers is written for use with a stock solution of 37% formaldehyde.

### 9.3 Preparation of Wash Buffer (0.1% Triton X-100 in PBS):

Prepare Wash Buffer by adding the provided 10% Triton X-100 solution to 1X PBS and mixing thoroughly.

### 9.4 Quenching Buffer (Wash Buffer containing 1% H<sub>2</sub>O<sub>2</sub> and 0.1% Sodium Azide):

Quenching Buffer is used to inactivate the cells' endogenous peroxidase activity. Prepare by adding fresh Sodium Azide and fresh hydrogen peroxide to the Wash Buffer.

### 9.5 Blocking Buffer:

Supplied ready-to-use. A small amount of white precipitate may form if thawed in a warm water bath. This does not interfere with buffer function.

### 9.6 Antibody Dilution Buffer:

Supplied ready-to-use. A small amount of white precipitate may form if thawed in a warm water bath. This does not interfere with buffer function.

**9.7 Diluted phospho-c-Src antibody:**

Prepare by diluting the supplied antibody 1/500 in Antibody Dilution Buffer (see the Quick Chart for Preparing Buffers).

**9.8 Diluted total-c-Src antibody:**

Prepare by diluting the supplied antibody 1/500 in Antibody Dilution Buffer (see the Quick Chart for Preparing Buffers).

**9.9 Diluted HRP-conjugated secondary antibody:**

Prepare by diluting the supplied antibody 1/2000 in Antibody Dilution Buffer (see the Quick Chart for Preparing Buffers).

**9.10 Preparation of Chemiluminescent Working Solution:**

The Chemiluminescent Reagent and Reaction Buffer should be warmed to room temperature before use. These components are light sensitive, therefore, we recommend avoiding direct exposure to intense light during storage. Prior to use, place the Chemiluminescent Reagent and Reaction Buffer at room temperature for at least 1 hour. In a separate container, mix 1 volume of Chemiluminescent Reagent with 2 volumes of Reaction Buffer to prepare the Chemiluminescent Working Solution (see the Quick Chart for Preparing Buffers in this section). The Chemiluminescent Working Solution is stable for several hours. After the Chemiluminescent Working Solution is aliquoted into the wells, discard the remaining solution.

**9.11 1% SDS Solution:**

Supplied ready-to-use. 1% SDS Solution is used in the Crystal Violet counting procedure to solubilize cells and release the dye for subsequent quantification at 595 nm.

**9.12 Crystal Violet Solution:**

Supplied ready-to-use. Crystal Violet is used to estimate the relative number of cells in each well. This stain binds to cell nuclei and gives an OD<sub>595</sub> reading that is proportional to cell number.

## Quick Chart for Preparing Buffers

Reagents to prepare	Components	1 well	48 wells	96 wells	192 wells
Fixing Buffer for adherent cells	1X PBS	98 $\mu$ L	4.7 mL	9.41 mL	18.82 mL
	37% Formaldehyde	12 $\mu$ L	576 $\mu$ L	1.15 mL	2.30 mL
Fixing Buffer for non-adherent cells	1X PBS	86 $\mu$ L	4.13 mL	8.26 mL	16.51 mL
	37% Formaldehyde	24 $\mu$ L	1.15 mL	2.30 mL	4.61 mL
Wash Buffer	1X PBS	3.376 mL	162 mL	310 mL	620 mL
	10% Triton X-100	34.1 $\mu$ L	1.64 mL	3.13 mL	6.26 mL
Quenching Buffer	Wash Buffer	97.9 $\mu$ L	4.7mL	9.40 mL	18.8 mL
	10% H <sub>2</sub> O <sub>2</sub>	11 $\mu$ L	528 $\mu$ L	1.06 mL	2.11 mL
	10% Sodium Azide	1.1 $\mu$ L	52.8 $\mu$ L	106 $\mu$ L	211 $\mu$ L
Diluted total-c-Src antibody	Antibody Dilution Buffer	45 $\mu$ L	2080 $\mu$ L	4160 $\mu$ L	-
	Total-c-Src antibody	0.09 $\mu$ L	4.16 $\mu$ L	8.32 $\mu$ L	-
Diluted phospho-c-Src antibody	Antibody Dilution Buffer	45 $\mu$ L	2080 $\mu$ L	4160 $\mu$ L	-
	Phospho-c-Src antibody	0.09 $\mu$ L	4.16 $\mu$ L	8.32 $\mu$ L	-
Diluted HRP-conjugated secondary antibody	Antibody Dilution Buffer	110 $\mu$ L	5280 $\mu$ L	10.56 mL	21.12 mL
	HRP-conjugated secondary antibody	0.055 $\mu$ L	2.64 $\mu$ L	5.28 $\mu$ L	10.56 $\mu$ L
1X PBS (for wash steps)	10X PBS	154 $\mu$ L	7.39 mL	14.11 mL	28.22 mL
	dH <sub>2</sub> O	1.39 mL	66.53 mL	127.01 mL	254.02 mL
Chemiluminescent Working Solution	Chemiluminescent Reagent	18 $\mu$ L	864 $\mu$ L	1.728 mL	3.46 mL
	Reaction Buffer	36 $\mu$ L	1.728 mL	3.456 mL	6.91 mL

## 10. Sample Preparation

### 10.1 Adherent cell protocol:

10.1.1 Seed cells in the 96-well plate so that they will be approximately 80% confluent at the time of fixing, after they have been treated as desired. The growth area in each well of the 96-well plate is 0.32 cm<sup>2</sup>. The provided plates are sterile and treated for tissue culture.

10.1.2 Grow and treat cells as desired.

10.1.3 Fix cells by replacing the growth medium with 100  $\mu$ L of 4% formaldehyde in PBS. To minimize the escape of formaldehyde vapors, place a 10 cm x 17 cm piece of parafilm over the plate and then cover the plate with the lid. The covered plate can also be placed in a zip-lock bag. Incubate for 20 minutes at room temperature.

**Δ Note:** Formaldehyde is highly toxic. Confine vapors to a chemical hood and wear appropriate gloves and eye protection when using this chemical.

### 10.2 Non-Adherent Cell Protocol:

The protocol is suitable for use with non-adherent cells if the cells are cultured and fixed as follows:

10.2.1 Treat the 96-well culture plate with 10  $\mu$ g/ml poly-L-Lysine for 30 minutes at 37°C. Wash twice for 5 minutes with PBS.

10.2.2 Seed 17,000 cells/well, or whatever amount is appropriate for your particular cell line.

10.2.3 Grow and treat cells as desired.

10.2.4 Fix cells by replacing the growth medium with 100  $\mu$ L of 8% formaldehyde in PBS. Incubate for 20 minutes at room temperature.

**Δ Note:** Fixed cells should be stored refrigerated in a zip-lock or heat-sealed bag with the formaldehyde solution in the wells.

## 11. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

### 11.1 Block cells

- 11.1.1 Remove formaldehyde solution and wash cells 3 times with 200  $\mu$ L Wash Buffer. Each wash step should be performed for 5 minutes with gentle shaking.
- 11.1.2 Remove Wash Buffer, add 100  $\mu$ L Quenching Buffer and incubate for 20 minutes at room temperature.
- 11.1.3 Remove Quenching Buffer and wash cells 2 times for 5 minutes each with 200  $\mu$ L Wash Buffer.
- 11.1.4 Remove Wash Buffer, add 100  $\mu$ L Antibody Blocking Buffer and incubate 1 hour at room temperature.

### 11.2 Binding of primary and secondary antibodies

**$\Delta$  Note:** Depending on experiment design, some wells may be incubated with diluted phospho-c-Src antibody, some with total-c-Src antibody and some with secondary antibody alone (negative controls). For negative control wells, incubate with 40  $\mu$ L Antibody Dilution Buffer during primary antibody incubation step.

- 11.2.1 Remove Antibody Blocking Buffer and wash cells 2 times with 200  $\mu$ L Wash Buffer.
- 11.2.2 Remove Wash Buffer, add 40  $\mu$ L of diluted primary antibody (or Antibody Dilution Buffer for negative control wells) and seal plate with sealing tape. Place a 10 cm x 17 cm piece of parafilm over the plate, cover with lid and incubate overnight at 4°C. Be sure that the plate is level and that each well is tightly sealed with the sealing tape to prevent evaporation.  
 **$\Delta$  Note:** In cells known to generate high amounts of phosphorylated c-Src, a 3-hour primary antibody incubation is sufficient. For maximum sensitivity, an overnight incubation is recommended.
- 11.2.3 Remove primary antibody, wash cells 3 times for 5 minutes each with 200  $\mu$ L Wash Buffer.

- 11.2.4 Remove Wash Buffer, add 100  $\mu$ L diluted secondary antibody, cover plate with tissue culture plate lid or sealing tape, and incubate 1 hour at room temperature.
- 11.2.5 During this incubation, place the Chemiluminescent Reagent and Reaction Buffer at room temperature.
- 11.3 **Chemiluminescent detection**
- 11.3.1 Remove secondary antibody, wash cells 3 times for 5 minutes with 200  $\mu$ L Wash Buffer and then 2 times for 5 minutes with 200  $\mu$ L 1X PBS.
- 11.3.2 Remove PBS from plate wells and add 50  $\mu$ L room temperature Chemiluminescent Working Solution to each well.
- 11.3.3 Read chemiluminescence using a luminometer or CCD camera system. Readings should be taken within 10 minutes to minimize changes in signal intensity.
- Δ Note:** The phospho-c-Src and total-c-Src antibodies can be used on equivalent cell cultures to determine the effects of various cell treatments on the ratio of phosphorylated c-Src to total c-Src. However, if the signals with the phospho-c-Src antibody and the total-c-Src antibody are identical, one cannot conclude that the treatment resulted in phosphorylation of 100% of the c-Src.
- 11.4 **OPTIONAL - Crystal Violet cell staining**
- Crystal Violet is an intense stain that binds to the cell nuclei and gives an OD<sub>595</sub> reading that is proportional to cell number. If you wish to normalize your readings from above, simply follow the steps below.
- 11.4.1 After reading chemiluminescence, wash wells twice with 200  $\mu$ L Wash Buffer and 2 times with 200  $\mu$ L 1X PBS. Tap plates onto paper towels to remove excess liquid from wells and air-dry at room temperature for 5 minutes.
- 11.4.2 Add 100  $\mu$ L Crystal Violet solution to each well and incubate 30 minutes at room temperature.
- Δ Note:** Crystal Violet is an intense stain. Avoid contact with skin and clothing.
- 11.4.3 Wash wells 3 times with 200  $\mu$ L 1X PBS for 5 minutes each.
- 11.4.4 Add 100  $\mu$ L of 1% SDS Solution to each well and incubate on shaker for 1 hour at room temperature.
- 11.4.5 Read absorbance on a spectrophotometer at 595 nm. If the signals obtained are greater than the range of your spectrophotometer, the signal can be reduced by removing

some (*e.g.* 50  $\mu\text{L}$ ) of the liquid from each well and replacing with an equivalent volume of  $\text{dH}_2\text{O}$ .

- 11.4.6 The measured  $\text{OD}_{595}$  readings indicate the relative number of cells in each well. This relative cell number is then used to normalize each reading from Step 11.3.

## 12. Assay Specificity

The phospho-c-Src antibody is specific for phosphorylated c-Src and was raised against a synthetic phospho-peptide corresponding to residues surrounding Tyrosine 418 of human c-Src. This antibody recognizes c-Src when phosphorylated at this site and also recognizes Fyn and c-Yes. The total-c-Src antibody recognizes c-Src proteins regardless of the phosphorylation state.

## 13. Species Reactivity

This kit recognizes phosphorylated c-Src from human, mouse and chicken origin and total c-Src from human and mouse origin.

## 14. Troubleshooting

Problem	Reason	Solution
<b>No signal or weak signal in wells incubated with either phospho-c-Src antibody or total-c-Src antibody.</b>	Omission of key reagent	Check that all reagents have been added in the correct order
	Substrate or conjugate is no longer active	Test conjugate and substrate for activity
	Enzyme inhibitor present	Sodium azide will inhibit the peroxidase reaction, follow our recommendations to prepare buffers
	Plate reader or CCD camera settings not optimal	Verify the wavelength (measurement mode) and filter settings in the plate reader
	Cells do not contain detectable levels of phospho c-Src and/or total c-Src	Use Western blotting to confirm that cells contain detectable levels of protein(s) of interest. If you do not require all of the included antibodies, they can be used in Colorimetric Western blotting at a 1/400 dilution for the total and a 1/1000 for the phospho antibody
	Insufficient number of cells were plated	Plate cells so that they are 80% confluent at time of fixing
	Cells did not adhere correctly to plate	Follow protocol for use of non-adherent cells
	Cells are not from correct origin	Refer to species reactivity information
	Excessive washing	Wash steps should be 5 minutes each
	Incubation of secondary antibody was too long	Incubate secondary antibody for 1 hour

<b>High background in all wells</b>	Measurement time too long	Reduce integration time or exposure time on luminometer or CCD camera
	Concentration of antibodies too high	Perform antibody titration to determine optimal working concentration. Start using 1/500 for the phospho- and the total-antibody and 1/2000 for the secondary antibody. The sensitivity of the assay will be decreased
	Inadequate washing	Ensure all wells are filled with Wash Buffer and follow washing recommendations
	Inadequate quenching or blocking	Ensure that quenching and blocking steps were performed according to the protocol
<b>Uneven signal development</b>	Incomplete washing of wells	Ensure all wells are filled with Wash Buffer and follow washing recommendations
	Well cross-contamination	Follow washing recommendations
<b>No signal or weak signal in wells incubated with phospho-c-Src antibody</b>	Cell culture conditions did not induce phosphorylation of c-Src	Perform Western blot with phospho-c-Src antibody to confirm that cells contain detectable levels of phosphorylated c-Src
<b>Antibody solution evaporates from well during overnight incubation with primary antibody</b>	Sealing tape was incorrectly applied	Ensure that each well is sealed when sealing tape is applied and ensure that the parafilm sheet covers the plate completely before the lid is placed on the plate. The plate can also be placed in a zip-lock or heat-sealed bag
<b>Insufficient sensitivity</b>	Antibody concentration incorrect	If the cells studied have very low levels of the protein of interest, the sensitivity of detection may be improved by increasing the concentration of primary antibody used and by minimizing the incubation volume. It is possible to

		perform the overnight incubation in as little as 25 $\mu$ L, however, this will make multichannel pipetting difficult and requires the plate be carefully sealed and incubated on a level surface. Alternatively, if the cells have easily detectable levels of the phosphorylated protein and the detection of small changes in phosphorylation is desired, sensitivity of the assay may be improved by decreasing the concentration of the phospho antibody used
<b>Poor precision</b>	Cross-well read through	The 96-well plates provided are designed to minimize signal cross-well contamination. If possible, do not use the phospho and total antibodies in adjoining wells. If this is not possible, use the total antibody at a higher dilution

# 15. Notes

## Technical Support

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