SAMPLE PREPARATION PROTOCOLS FOR KINOMEVIEW™ ANALYSIS BY CELL SIGNALING TECHNOLOGY, INC.

CONTACT INFORMATION

PTMSCAN® SCIENTISTS:

Matthew Stokes, Ph.D.
Cell Signaling Technology, Inc.
3 Trask Lane, Room 2406
Danvers, MA 01923
978-867-2467; mstokes@cellsignal.com

Hongbo Gu, Ph.D.
Cell Signaling Technology, Inc.
3 Trask Lane, Room 2406
Danvers, MA 01923
978-867-2300, x2211; hongbo.gu@cellsignal.com

Charles Farnsworth, Ph.D.
Cell Signaling Technology, Inc.
3 Trask Lane, Room 2406
Danvers, MA 01923
978-867-2376; cfarnsworth@cellsignal.com

PROTEOMICS SERVICE MANAGER:

Jeffrey C. Silva, Ph.D.
Cell Signaling Technology, Inc.
3 Trask Lane, Room 2309
Danvers, MA 01923
978-826-6073; jsilva@cellsignal.com

PTMSCAN® PROTEOMICS SERVICE WEBSITE:

http://www.cellsignal.com/services/index.html

PHOSPHOSITEPLUS®:

http://www.phosphosite.org/homeAction.do

Sample Submission Form for QUOTE # _____		
Sample #	**Species**	**Description**
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NOTES:		

Print multiple sheets if the total number of samples exceeds twenty-eight or recreate the form in Excel for electronic submission/preparation. Modify numbering of additional sheets accordingly.

SHIPPING INSTRUCTIONS FOR KINOMEVIEW™ SAMPLES

All samples must be shipped to CST on dry ice by overnight courier.

NOTE: DO NOT ship any samples on a Thursday or Friday in order to prevent weekend holdover in transit and possible damage to valuable samples. It is preferable to ship materials on Monday or Tuesday.

Please notify by email the Proteomics Service Manager at jsilva@cellsignal.com prior to overnight shipment. Include in the email communication, the overnight courier and tracking number to ensure efficient delivery. Please include in your email a description of the samples as they are labeled on the tubes. It is preferable that you label the tubes with numbers or letters and provide a hard copy word file with the full description for each sample number/letter (see next page for Sample Submission Form). Also, please enclose a printed copy of the Sample Description Form with the overnight shipment. Please label the side of the box with your CST contract number. You will receive an email confirmation upon sample receipt.

NOTE: Please label the side of the shipping box with your CST contract number (QUOTE #)

SHIP KINOMEVIEW™ & PTMSCAN® SAMPLES TO:

Jeffrey C. Silva
Proteomics Service Manager
Cell Signaling Technology, Inc.
3 Trask Lane
Danvers, MA 01923
978-826-6073
jsilva@cellsignal.com

KINOMEVIEW™ SAMPLE PREPARATION PROTOCOL

NOTE: After reviewing the sample preparation document, please schedule a teleconference with the Proteomics Service Manager (Jeffrey Silva) to address any questions you may have with regard to the protocol and to help finalize the details of your KinomeView™ study (see contact information on page 2).

A. Solutions and Reagents:

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. Phosphate Buffered Saline (PBS-20X) (CST#9808): 1X: 137mM NaCl, 2.7mM KCl, 4.3 mM $Na_2HPO_4 \cdot 7H_20$, 1.4mM KH_2PO_4, pH 7.2
2. Cell Lysis Buffer (10X) (CST#9803)
3. Complete Mini EDTA-free Protease Inhibitor (Roche #11 836 170 001)

To prepare 1X Cell Lysis Buffer*:

a. Dissolve one protease inhibitor tablet in 7 mls Milli-Q water
b. Add 1 ml CST 10X cell lysis buffer

*The final volume is 8 mls to adjust for cell volume in the extract.

B. A general protocol for (~1 $x10^7$) adherent cell extract preparation is described below:

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X cold PBS; aspirate.
3. Lyse cells by adding 1X cold CST cell lysis buffer (600 μL per 15 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Microcentrifuge for 10 minutes, at 12,000 RPM, place supernatant in new microcentrifuge tube.
6. Flash freeze extracts on dry ice, and store at -80°C.

C. For suspension cells following treatment harvest by low speed centrifugation, wash 1X cold PBS, spin, aspirate PBS and flash freeze pellet on dry ice.

Harvest sufficient cells for 1 mg total protein (generally about 1-2$x10^7$ cells, depending on cell type). The sample requirements for tissue is approximately 50 mg (wet weight). Tissue can be prepared by flash-freezing the material in liquid nitrogen and placed a 2 mL screw cap vial.

Send cell pellet (or tissue) to CST on dry ice.