SignalScan™ Peptide Mix (SARS-CoV-2)

96 µl
(96 assays)

Cell Signaling TECHNOLOGY®

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rev 04/09/21

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Description: The SignalScan™ Peptide Mix (SARS-CoV-2) enables targeted analysis of selected peptides in human-sourced cells or tissues. Peptides and phosphopeptides derived from SARS-CoV-2 spike and nucleocapsid proteins as well as nine human host response proteins are included. This mixture contains 96 pmol each of 25 heavy-labeled (13C, 15N) Stable Isotope Standard (SIS) peptides, quantified by amino acid analysis (AAA). Inclusion of SIS peptides enhances the sensitivity of the LCMS assay by acting as a trigger for targeted MS/MS analysis. Targeted assays ensure that peptides present in the biological sample of interest are consistently observed and sensitivity is maximized. SIS-assisted scanning provides the sensitivity of a parallel reaction monitoring (PRM) assay without use of rigid retention time windows. Full peptide list, detailed protocol, and files for method set-up and data analysis are available for download at http://media.cellsignal.com/www/zip/proteomics/signalscan-peptide-mix.zip.

Background: The cause of the COVID-19 pandemic is a novel and highly pathogenic coronavirus, termed SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2). SARS-CoV-2 is a member of the Coronaviridae family of viruses (1). The genome of SARS-CoV-2 is similar to other coronaviruses, and is comprised of four key structural proteins: S, the spike protein, E, the envelope protein, M, the membrane protein, and N, the nucleocapsid protein (2). Coronavirus spike proteins are class I fusion proteins and harbor an ectodomain, a trans-membrane domain, and an intracellular tail (3,4). The highly glycosylated ectodomain projects from the viral envelope surface and facilitates attachment and fusion with the host cell plasma membrane. The ectodomain can be further subdivided into host receptor-binding domain (RBD) (S1) and membrane-fusion (S2) subunits, which are produced upon proteolysis by host proteases at S1/S2 and S2' sites. S1 and S2 subunits remain associated after cleavage and assemble into crown-like homotrimers (2,4). In humans, both SARS-CoV and SARS-CoV-2 spike proteins utilize the angiotensin-converting enzyme 2 (ACE2) protein as a receptor for cellular entry (5-7). The cell's subsequent host response to SARS-CoV-2 infection involves complex signaling cascades, observable through perturbations in protein phosphorylation patterns (8,9) that lead to interferon and cytokine production (10).

Protein	PTM	Peptide
Spike		TQLPPAYTNSFT**R**
Spike		VGGNYNYLY**R**
Spike	R815 uncleaved	RSFIEDLLFN**K**
Spike		SFIEDLLFN**K**
Spike		LIANQFNSAIG**K**
Nucleocapsid		RPQGLPNNTASWFTALTQHG**K**
Nucleocapsid		ADETQALPQ**R**
Nucleocapsid		GFYAEGS**R**
Nucleocapsid	pS176	GFYAEGS***R**
Nucleocapsid	pT198	NST*PGSS**R**
Nucleocapsid	pS206	GTS*PA**R**
ACE2		GDYEVNGVDGYDYS**R**
ACE2		NSFVGWSTDWSPYADQSI**K**
IL6		EALAENNLNLP**K**
IL6		YILDGISAL**R**
IL8		ELCLDP**K**
Interferon alpha		SLSLSTNLQE**R**
Interferon beta		LLWQLNG**R**
Jak1	pY1034	AIETDKEY*YTV**K**
Jak1	pY1034/pY1035	AIETDKEY*Y*TV**K**
STAT1	pS727	LQTTDNLLPMS*PEEFDEVS**R**
STAT1	pY701	GTGY*IKTELISVSEVHPS**R**
IRAK4	pT345	FAQTVMT*S**R**
IRAK4	pT345/pS346	FAQTVMT*S***R**
PERK	pS715	EHIEIIAPS*PQ**R**

Storage: This product is stable for 12 months when stored at -20°C. *Aliquot to avoid multiple freeze/thaw cycles.*

Background References:

(1) Zhou, P. et al. (2020) *Nature* 579, 270-273.

(2) Tortorici, M.A. and Veesler, D. (2019) *Adv Virus Res* 105, 93-116.

(3) Li, F. et al. (2006) *J Virol* 80, 6794-800.

(4) Li, F. (2016) *Annu Rev Virol* 3, 237-261.

(5) Shang, J. et al. (2020) *Nature* 581, 221-224.

(6) Wrapp, D. et al. (2020) *Science* 367, 1260-1263.

(7) Yan, R. et al. (2020) *Science* 367, 1444-1448.

(8) Davidson, A.D. et al. (2020) *Genome Med* 12, 68.

(9) Bouhaddou, M. et al. (2020) *Cell* 182, 685-712.e19.

(10) Blanco-Melo, D. et al. (2020) *Cell* 181, 1036-1045.e9.

◄ *Peptides included in SignalScan™ Peptide Mix (SARS-CoV-2). Peptides in blue are derived from SARS-CoV-2 proteins. Peptides in black represent human proteins. All peptides are stable-isotope labeled on their carboxy-terminal amino acid ($^{15}N_4$$^{13}C_6$-Arg or $^{15}N_2$$^{13}C_6$-Lys), designated by bold **R** or **K**. Phosphorylated residues are designated with an asterisk (*).*
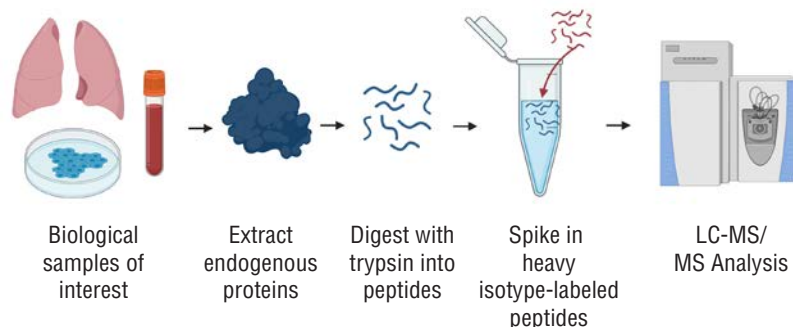
◄ *SignalScan™ Workflow Steps. The SignalScan™ Peptide Mix (SARS-CoV-2) is compatible with any human sourced biological material. Extract proteins from the tissue and digest them into peptides using any standard trypsin-based protocol. Spike in 1 pmol of the heavy Stable Isotope Standard (SIS) peptides provided prior to LC-MS/MS analysis.*

Biological samples of interest → Extract endogenous proteins → Digest with trypsin into peptides → Spike in heavy isotope-labeled peptides → LC-MS/MS Analysis

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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SignalScan™ Peptide Mix (SARS-CoV-2) Protocol

A Product Provided

SignalScan™ Peptide Mix (SARS-CoV-2) at a concentration of 1 pmol/µl per peptide in 0.1% trifluoroacetic acid, 25% acetonitrile. Peptides are labeled with 13C, 15N at carboxy-terminal lysine or arginine residues and are quantified by amino acid analysis.

B Downloadable Analysis Package Contains

- Peptide list
- SignalScan™ Stable Isotope Standard Assisted Scanning (SISAS) protocol
- SureQuant method screenshots
- Skyline templates
- Survey Run_Targeted Mass.csv
- SureQuant_Targeted Mass_Intensity.csv example files (four files: K2+, K3+, R2+, R3+)
- SureQuant_Targeted Mass_Trigger.csv (four files: K2+, K3+, R2+, R3+)

C Sample Preparation and Analysis

- Dilute SignalScan™ Peptide Mix (SARS-CoV-2) at least 5x in your LC-MS loading buffer (not provided, 0.1% TFA recommended) prior to LC-MS injection to reduce the acetonitrile concentration.
- For SureQuant analysis, dilute 1 pmol SignalScan™ Peptide Mix (SARS-CoV-2) into 1-2 ug purified peptide sample (from tissue, cell lysate, or other sample digested with trypsin) for each LC-MS injection.
- For details of SureQuant method LCMS setup and data analysis, download analysis package from http://media.cellsignal.com/www/zip/proteomics/signalscan-peptide-mix.zip and follow instructions in SignalScan™ protocol.