



Association of Biomolecular Resource Facilities
2019 Galisteo Street, Bldg. I, Santa Fe, NM 87505

sPRG2007 Phosphorylation Study ♦ Email: mag4@columbia.edu

Re: Proteomics Standards Research Group sPRG2007 Phosphorylation Standard Study

Dear sPRG07 Study Participant,

Thank you for participating in the Proteomics Standards Research Group sPRG2007 Study. We know many of you are anxious to enter your results and we expect to open the results survey this Mon 15 Jan.

SurveyMonkey does not provide a facile way to capture spreadsheet-like, tabular information. In order to ensure that we can properly parse and understand your phosphopeptide sequence results, we are reiterating the data entry format instructions found in the sPRG07 survey.

We strongly recommend you create the entire properly formatted phosphopeptide list in a backup document and then cut & paste it into the entry field to avoid any data loss.

The phosphopeptides for all phosphoproteins you detected will be entered in the general format:

Accession1, EntryName1, Protein1Name (for the first phosphoprotein)

PhosphoPeptide Sequence 1(# of sites)
PhosphoPeptide Sequence 2(# of sites)
PhosphoPeptide Sequence 3(# of sites)
etc.

Accession2, EntryName2, Protein2Name (for the second phosphoprotein)

PhosphoPeptide Sequence 4(# of sites)
PhosphoPeptide Sequence 5(# of sites)
etc.

next phosphoprotein etc...

Specific Instructions: To capture your phosphopeptide results properly please follow the formatting instructions *on the following page exactly* ➔.

Thank you.

ABRF Proteomics Standards Research Group

Enter your results at: <http://www.surveymonkey.com/s.asp?u=994683019041>

Instructions for Reporting Phosphopeptides in the sPRG2007 Study Survey

A. First, begin each proteins' phosphopeptide list with a single line, listing the: SwissProt accession number (P00000), entry name (XXXXXX_HUMAN), and protein name (ABRFPRGase).

B. Next, for each phosphopeptide, the sPRG needs to capture four items:

- the phosphopeptide sequence
- the number of phosphates on each phosphopeptide you detected
- the position(s) in the phosphopeptide
- whether the position of phosphorylation is precise or ambiguous

To accomplish this, please list phosphopeptides below **exactly** as follows:

- **Only ONE phosphopeptide per line**
- **Sequence:** Write the phosphopeptide sequence **in single-letter amino acid code in CAPITAL letters**, e.g. A,C,D,E, etc only.
- **Number:** Indicate the number of phosphates on the observed peptide in parentheses, e.g. "(2)", immediately following the C-terminal residue.
- **Position:** For phosphorylation sites whose position is high confidence, precede the phosphorylated residue with a lowercase "p" e.g. AADDpTSWEPFASGK(2). (This example indicates a doubly phosphorylated peptide, T5 AND S12 are BOTH phosphorylated.)
- **Confidence of position:** If a phosphorylated peptide is detected but the precise position of a phosphate cannot be determined, precede the ambiguous positions with a lowercase "x", e.g. AADDxTxSWEPFASGK(1). (This example indicates a singly phosphorylated peptide, on T5 OR S6, but not S12.)
[Note: phosphopeptides determined by mass only, such as a MALDI-TOF mass map, would therefore have ALL ser, thr, tyr preceded by a lowercase "x" since the number of phosphates would be known but not their position, e.g AADDxTxSWEPFASGK(1).]
- **List all unique detections.** For example if you have separate spectra for the singly phosphorylated peptides AADDpTSWEPFASGK(1) and AADDTSWEPFASGK(1), they should be listed on separate lines and not as AADDpTSWEPFASGK(2).

Your final inputted phosphoprotein/peptide list should look as follows:

P00000, XXXXX_BOVIN, Calf muscle ABRFase

AADDTSWEPFASGK(1)
AADDpTSWEPFASGK(1)
YTEHNNxSGxTK(1)
GGVIpTAEILHFNxTxTR(2)

P00001, YYYYYY_HUMAN, sPRGetin

EFFpTKWALAK(1)
MARYxSGxANNGAWR(1)
WILpTIAMxTxTLANR(2)