



Association of Biomolecular Resource Facilities *Proteomics Standards Research Group (sPRG):*

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sPRG2018 Study Sample Request

The mission of the ABRF proteomics Standards Research Group (sPRG) is to design and develop standards and resources for mass-spectrometry-based proteomics experiments. Recent advances in methodology have made phosphopeptide analysis a tractable problem for core facilities. We constructed a pool of heavy-labeled phosphopeptides that will enable core facilities to rapidly develop assays. Our pool contains 150 phosphopeptides that have previously been observed in mass spectrometry data sets. The specific peptides were chosen to cover as many known biologically interesting phosphosites as possible from seven different signaling pathways: AMPK signaling, death and apoptosis signaling, EGFR/HER signaling, insulin/IGF-1 signaling, mTOR signaling, PI3K/AKT signaling, and stress (p38/SAPK/JNK) signaling. We feel this pool will enable researchers to test the effectiveness of their enrichment workflows and to provide a benchmark for a cross-lab study.

For this year's study, we invite participants to use this controlled standard in a tryptic HeLa cell lysate background to evaluate their phosphoproteomic data acquisition and analysis workflows. We hope this standard is helpful in a number of ways, including enabling phosphopeptide sample workflow development, as an internal enrichment and chromatography calibrant, and as a pre-built biological assay for a wide variety of signaling pathways.

Laboratories requesting samples will receive two (2) vials:

- 1) One vial containing a mixture of approximately 2pmol of each standard peptide (dried).
- 2) One vial containing the standard peptides mixed with 1mg of a tryptic digest of EGF and IGF1 activated HeLa cell lysate (dried) → note: no digestion required.

Participants are asked to enrich the phosphopeptides from sample 2 with their preferred method (either TiO₂ or Fe, or any other that you are currently using) and run the two samples on your LC-MS system of choice with the same method.

In addition to providing endogenous-to-standard peptide abundance ratios using a common spreadsheet, we are also asking for the participants to send us the raw mass spectrometry files. Further instructions will be sent with the samples once requests have been received.

This year's study is once again open to both ABRF members and non-members. However, the total number of samples is limited, and priority will be given to ABRF members. Non-members are encouraged to join the ABRF (for more information, go to <http://www.abrf.org>).

Vendors/sponsors are welcome to participate. **Each laboratory interested in participating should only request one sample per lab.**

Sample requests must be submitted by email to abrfSprg2018@gmail.com prior to December 20th, 2017. Please include the words "Sample request" in the subject line of the message and provide a contact name, affiliation, and complete mailing address in the body of the message. To reiterate, because of limited availability and the significant effort involved in preparation of the samples by the sPRG, the Research Group asks that samples only be requested if there is a reasonable probability that you will be able to return data by the deadline. **Samples will be sent out in the first week of January, and we expect results to be returned by February 12th.** As in the past, **result submissions will be coded to insure anonymity of the participating laboratories so we encourage everyone to participate, no matter the experience level.** A summary of the results of this study will be presented orally and as a poster at the ABRF 2018 meeting; the results will be subsequently posted on the ABRF website and will ultimately be published. We thank you for your support of the ABRF. We look forward to your participation in this study and your help developing this standard.

Sincerely,

The ABRF Proteomics Standards Research Group

Antonius Koller (Chair) - Columbia University
Christopher Colangelo (ad hoc) - Primary Ion
David Hawke - UT MD Anderson Cancer Center
Gordana Isovev - Sciex
Kimberly A. Lee - Cell Signaling Technology
Ryan Leib - Stanford University
Bhavin Patel - Thermo Fisher Scientific
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