

# PhosCalc 1.2 (Standalone Version)

Requirements: Perl, Math::Combinatorics Module  
(<http://search.cpan.org/~allenday/Math-Combinatorics-0.08/lib/Math/Combinatorics.pm#AUTHOR>)

## About PhosCalc

PhosCalc is a system for estimating the site of phosphorylation in a phosphorylated peptide with ambiguous sites.

It does this by generating ions from peptide sequences and calculating their masses, generating different ions and masses for water-loss, different cysteine modifications and different charge states. These are then compared with peaks in the spectrum from which the peptide was identified to assess the most likely site of p. The process is carried out as described in [MacLean et al](#)

## Feature additions over version 1:

- will now accept mgf file
- will add water-loss ions to predicted set
- will deal with different charge states
- allows selection of masses for different cysteine modifications
- assesses calculated probabilities of combinations of phosphorylation sites to produce a best guess formatted string

## Running PhosCalc:

### Quick Start

#### Input

As a minimum PhosCalc requires a peptide spectrum list file and spectrum files to run.

The spectra may be either .dta or .mgf format. Default is .dta.

The peptide spectrum list must contain each peptide to be assessed and the name of the corresponding spectrum. The info should be in two columns, the peptide on the left and its spectrum on the right, separated by a tab character

eg THISISAPEPTIDE      aspectrumfile.dta

If you like you can build the peptide list file in excel and save it as a 'tab delimited' file.

If you are using .dta files, the spectrum column should contain the name of the .dta file, if you are using .mgf then the spectrum column should contain the name of the spectrum as given in the TITLE= line.

## To run PhosCalc

### Using .dta files

Put the peptide spectrum list file and the .dta files in the same folder as the script

From the command line simply type `perl phoscalc1.2.pl -p <peptide spectrum list>`

PhosCalc will run with

experiment type MS2

error margin of 0.4

filtering the four strongest peaks from each 100 m/z window of the spectrum using a best guess range of 3 orders of magnitude (1000)

output will go into a file with the name of your peptide spectrum list appended with '\_phoscalc\_output.tab'

### Using .mgf files

Put the spectrum list file and the .mgf file in the same folder as the script.

From the command line type `phoscalc1.2.pl -p <peptide spectrum list> -t mgf -m <mgf file name>`

PhosCalc will run with

experiment type MS2

error margin of 0.4

filtering the four strongest peaks from each 100 m/z window of the spectrum using a best guess range of 3 orders of magnitude (1000)

output will go into a file with the name of your peptide spectrum list appended with '\_phoscalc\_output.tab'

### Other settings

You can customise the behaviour of PhosCalc using the command line flags. To see a summary of these type `phoscalc1.2.pl -h` at the command line.

`-p <some file>` defines the peptide spectrum list file

`-t <'dta' or 'mgf'>` defines the spectrum file type (defaults to dta if omitted)

`-m <some file>` defines the .mgf file (required if -t is set)

`-x` defines the experiment type (defaults to MS2 if omitted)

`-e` defines the width of window for peak matching. (defaults to 0.4 if omitted)

- D calculate additional ions with loss of water
- M use carboxmethylated cysteine value (mutually exclusive with -A)
- A use carboxamidomethylated cysteine (mutually exclusive with -M)

unmodified cysteine mass = 103.00919

alkylated cysteine (carboxmethylation) = 103.00919 + 58.00548

alkylated cysteine (carboxamidomethylation) = 103.00919 + 57.021464

-o <some file> defines the output file (defaults to input\_file\_phoscalc\_output.tab if omitted)

-b <a number> defines the range of p values over which to consider a phosphorylation site equivalent for best guess formatting. eg 10 = 1 order of magnitude, 1000 = 3 orders of magnitude, 1000000 = 6 orders of magnitude. (defaults to 1000 if omitted).

-h print the help and quit

### References

MacLean D, Burrell MA, Studholme DJ and Jones AMEJ (2008) PhosCalc: A tool for determining the likelihood of peptide phosphorylation from Mass Spectrometer data. *BMC Research Notes* .1:30 ([PubMed](#))