Minimal Information About Sample Preparation for Phosphoproteomics

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This module identifies the minimum information required to report sample preparation conditions in a phosphoproteomics experiment according to the MIAPE Principles.

Introduction

In the study of the phosphoproteome, sample preparation for peptide or protein enrichment is an especially critical step. In most cases, this is achieved using a combination of procedures including HPLC separation of peptides or proteins, gel electrophoresis and enrichment methods such as immunoaffinity or IMAC and TiO2-based purification procedures.

This guideline describes parameters and conditions involved in phosphopeptide identification by mass spectrometry. It covers from the description and preparation of the cells and tissues to the fractionation and specific enrichment of phosphopeptides for MS analysis. The guideline is prepared in order to easily cope with many of the experimental designs used in phosphoproteomic studies.

The document is subdivided as follows:

1. General features
2. Sample processing
3. Protein Purification/Fractionation
4. Peptide Purification/Fractionation
5. Phosphopeptide enrichment

If procedures are repeated more than once, the corresponding section should be repeated (e.g. More than one purification method for phosphopeptide enrichment).

In sections where the methodology described has been already documented as part of other MIAPEs (1D SDS-PAGE and HPLC purifications), the corresponding sections have been imported from them. Thus, this proposal includes some definitions from HUPO-PSI Gel Electrophoresis vs. 1.4, January 2008 and Column Chromatography vs. 1.0, June 2008 documents.

Reporting requirement for Sample Preparation for Phosphoproteomics

1. General features

1.1 Date stamp (as yyyy-mm-dd)

1.2 Responsible person or institutional role

1.3 Sample/s name/s

1.4 Replicates

2. Sample processing

2.1 Culture/tissue handling

- Tissue/Cell/Fluid type
- Source
- Species
- Isolation/Collection Method

2.1.1 Cells

2.1.1.1 Cell Isolation Method

2.1.1.2 Culture parameters

- Culture medium
- Culture conditions
- Cell density
- Type of culture
- Time of culture

2.1.1.3 Cell treatment

- Starting amount
- Culture medium
- Culture conditions
- Cell Treatment
- Cell density
- Time

2.1.1.4 Pellet preparation

2.1.1.5 Cell/Pellet lysis

- Starting amount
- Lysis buffer
- Phosphatase inhibitors used
- Other enzyme inhibitors
- Volume
- Homogenization or sonication methods
- Centrifugation

2.1.1.6 Other procedures

2.1.2 Solid Tissues

2.1.2.1 Tissue disruption

- Starting amount
- Lysis buffer
- Phosphatase inhibitors used
- Other enzyme inhibitors
- Volume
- Homogenization or sonication methods
- Centrifugation

2.1.2.2 Other procedures

2.1.3 Fluids

- Starting volume
- Buffer used for dilution
- Phosphatase inhibitors used
- Other enzyme inhibitors
- Protein deplection method (if required)

2.2 Protein Precipitation

- Starting volume
- Reagents and conditions
- Centrifugation
- Redisolution

2.3 Protein Digestion

- Starting amount

2.3.1 Pretreatment

- Reduction
- Alkykation
- Others

2.3.2 Digestion parameters

- Protein concentration
- Digestion buffer
- Enzyme
- Incubation parameters

2.4 Peptide/Protein Labeling

- Type of labeling
- Significative customizations

2.5 Other treatments

3. Protein Purification/Fractionation

3.1 1D SDS-PAGE and in-gel digestion
- Starting material

3.1.1 Gel matrix and electrophoresis
- Description of gel matrix
- Gel manufacturer
- Physical dimensions
- Physicochemical property range and distribution
- Acrylamide concentration
- Acrylamide:crosslinker ratio
- Gel lane
- Sample application
- Buffer
- Equipment
- Electrophoresis conditions

3.1.2 Staining

3.1.3 In gel digestion
- Digestion buffer
- Enzyme
- Incubation parameters
- Previous treatment
- Automatic digestor

3.2 HPLC and liquid digestion
- Starting material

3.2.1 Equipment

3.2.1.1 Product details for column
-Make
-Model
-Separation mode

3.2.1.2 Physical characteristics of column
-Length
-Diameter
-Description of stationary phase

3.2.1.3 Chromatography system used for separation, where applicable

3.2.1.4 Mobile phase: for each mobile phase
-Name of mobile phase
- Description of the constituents

3.2.1.5 Properties of the column run
-Time
-Gradient

-Flow rate

-Temperature

-Separation purpose

3.2.1.6 Column outputs - (a) detection

- Equipment used for detection
- Type
- Equipment settings
- Timescale over which data was collected
- Trace

3.2.1.7 Column outputs - (b) fractions (if required)

- Fraction name
- Fraction description

3.2.2 Liquid digestion of fractions, if required

- Digestion buffer
- Enzyme
- Incubation parameters
- Previous treatment

3.2.3 Other parameters

4. Peptide Purification/Fractionation

4.1 HPLC

- Origin of the sample

4.1.1 Equipment

4.1.1.1 Product details for column

- Make
- Model
- Separation mode

4.1.1.2 Physical characteristics of column

- Length
- Diameter
- Description of stationary phase

4.1.1.3 Chromatography system used for separation

4.1.2 Mobile phase: for each mobile phase

- Name of mobile phase
- Description of the constituents

4.1.3 Properties of the column run

- Time
- Gradient
- Flow rate

- Temperature
- Separation purpose

4.1.4a Column outputs - (a) detection
- Equipment used for detection
- Type
- Equipment settings
- Timescale over which data was collected
- Trace

4.1.4b Column outputs - (b) fractions (if required)
- Fraction name
- Fraction description

4.1.5 Other parameters

5. Phosphopeptide Enrichment (Repeat the steps as necessary)

5.1 Method 1
- Method description

5.1.1 Starting sample
- Origin of the sample
- Volume
- Pretreatment
- Loading buffer

5.1.2 Affinity material
- Description of the affinity material
- Pretreatment of the affinity material

5.1.3 Affinity purification procedure
- Volume of sample
- Amount of affinity material
- Type of purification
- Incubation time
- Incubation temperature
- Washing buffer
- Elution buffer
- Name of the obtained fractions

5.1.4 Other parameters

Appendix I. The MIAPE Sample Preparation for Phosphoproteomics glossary of required-terms

	Classification	*Definition*
1. General features		
1.1	**Date stamp (as yyyy-mm-dd)**	The date on which the work described was initiated; given in the standard yyyy-mm-dd format (with hyphens)
1.2	**Responsible person or institutional role**	The (stable) primary contact person for this data set: this could be the experimenter, lab head, line manager, etc. Where responsibility rest with an institutional role (e.g. One of a number of duty officers) rather than a person, give the official name of the role rather than any one person. In all cases give affiliation and stable contact information, which consists of (i) Name, (ii) Postal address and (iii) Email address.
1.3	**Sample/s name/s**	Name of the samples processed. Include if there are control, standard of test samples and if samples are a pool of different individual.
1.4	**Replicates**	Number of replicates of the experiment.
2. Sample Processing		
2.1	**Culture/tissue handling**	
	Tissue/Cell/Fluid type	Biological material used in the experiment.
	Source	Source from which the material is obtained (e.g. T-cells from buffy coat)
	Species	Species of procedence
	Isolation/Collection Method	Surgical procedure, laser capture, intravenous blood extraction, etc
2.1.1	**Cells**	
2.1.1.1	**Cell Isolation Method**	Method used for cell isolation (e.g. Ficoll-Paque T-cells purification from buffy coat, enzymatic digestion, explant culture...)
2.1.1.2	**Culture parameters**	
	Culture medium	Growth medium with all the complements added
	Culture conditions	Temperature and gas mixture (typically for mammalian cells, 37ºC, 5% CO_2)
	Cell density	Number of cells per mL
	Type of culture	Indicate if there are suspension or adherent cells and if they are primary cultures
	Time of culture	Time which the cells are in culture and number of previous passages.
2.1.1.3	**Cell treatment**	
	Starting Amount	Number of cells submitted to treatment
	Culture medium	Culture medium for activation with all the complements added
	Culture conditions	Temperature and gas mixture used for treatment
	Cell Treatment	Cell treatment (activator,drug). Include concentration and buffers used for addition

	Cell density	Number of cells per mL during treatment
	Time	Time for which the cells are treated.
2.1.1.4	**Pellet preparation**	Procedure used to prepare the pellet (wash, centrifugation or other parameters)
2.1.1.5	**Cell/Pellet lysis**	
	Starting amount	Number of cells submitted to lysis.
	Lysis buffer	Buffer used for cell lysis (Indicate all the components, concentration and pH).
	Phosphatase inhibitors used	Name, manufacturer, reference, composition and amount added to the buffer.
	Other enzyme inhibitors	Name, manufacturer, reference, composition and amount added to the buffer.
	Volume	Volume of buffer used for lysis (in SI units).
	Homogenization or sonication methods	Method used for cell/tissue disruption, include the time and power of the homogenization or sonication process.
	Centrifugation	Centrifugation time and centrifugal force to obtain a clarified protein solution
2.1.1.6	**Other procedures**	Other procedures or treatments performed in cell preparation not included in 2.1.1.1.to 2.1.1.5
2.1.2	**Solid Tissues**	
2.1.2.1	**Tissue disruption**	
	Starting Amount	Mass in SI units of starting material. Indicate whether it is wet or dry weight
	Lysis buffer	Buffer used for tissue disruption (Indicate all the components, concentration and pH).
	Phosphatase inhibitors used	Name, manufacturer, reference, composition and amount added to the buffer
	Other enzyme inhibitors	Name, manufacturer, reference, composition and amount added to the buffer.
	Volume	Volume of buffer used for lysis (in SI units).
	Homogenization or sonication methods	Method used for tissue disruption. Include the time and power of the homogenization or sonication process.
	Centrifugation	Centrifugation time and centrifugal force
2.1.2.2	**Other procedures**	Other procedures or treatments performed in tissue preparation not included in 2.1.2.1.
2.1.3	**Fluids**	
	Starting Volume	Volume of initial fluid used for the analysis (in SI units)
	Buffer used for dilution	Buffer used for fluid dilution prior to protein depletion, digestion or other procedure (Indicate all the components, concentration and pH). .
	Phosphatase inhibitors used	Name, manufacturer, reference, composition and amount added to the sample.
	Other enzyme inhibitors	Name, manufacturer, reference, composition and amount added to the buffer.
	Protein deplection method (if required)	If required indicate the method of protein depletion, the manufacturer and any significative customization from the original procedure.
2.2	**Protein Precipitation**	

	Starting Volume	Volume of sample used for protein precipitation (in SI units)
	Reagents and conditions	Reagents used for precipitation. Indicate the composition and the volume added to the sample (in SI units).
	Centrifugation	Centrifugation time and centrifugal force.
	Redissolution	Buffer used to dissolve the precipitated proteins. Indicate the composition and the volume added to the pellet (in SI units). Also indicate if additional procedures as sonication or incubation are used to increase protein solubilization.
2.3	**Protein Digestion**	
	Starting Amount	Mass in SI units of starting material.
2.3.1	**Pretreatment**	
	Reduction	Reagents, time, temperature and concentration.
	Alkykation	Reagents, time, temperature and concentration
	Others	Other treatments before digestion. Include reagents, time, temperature and concentration.
2.3.2	**Digestion parameters**	
	Protein concentration	Concentration of protein extract submitted to digestion.
	Digestion buffer	Buffer used for digestion. Indicate components, concentration and pH
	Enzyme	Enzyme description (solubilization buffer and concentration).
	Incubation parameters	Incubation time and temperature
2.4	**Peptide/Protein Labeling**	
	Type of labeling	Type and make of the labeling reactive (e.g. iTRAQ from Applied Biosystems)
	Significative customizations	Any significant deviations from the manufacture's protocol
2.5	**Other treatments**	Other procedures or treatments performed in the sample preparation not included in 2.1.to 2.4
3. Protein Purification/Fractionation		
3.1	**1D SDS-PAGE and in-gel digestion**	(From HUPO-PSI MIAPE:Gel Electrophoresis vs 1.4, January 2008)
	Starting Material	Mass in SI units (cells or tissues) or volume of fluid submitted for separation.
3.1.1	**Gel matrix and electrophoresis**	
	Description of gel matrix	Gel matrix being used. Include the descriptive name of the matrix (e.g. IPG strip, slab gel) and the type of the matrix used (e.g. a native gel, denaturing gel, gradient gel, etc.). State whether the matrix is composed of more than one kind of gel and name the parts (e.g. stacking gel). Give the dimensions of the matrix and associated parts, under the physical dimension section, below.

	Gel manufacturer	If the gel was purchased pre-cast, then include the model name, model number, batch number and manufacturer. If the gel has been manufactured 'in house' then a reference to published protocol should be given. If no published protocol is available a recipe should be given.
	Physical dimensions	The physical dimensions of the gel matrix. The measurements must be in the form of the Cartesian Coordinate system (x,y,z). According to the standard image orientation described in section 7.1.6, x represents the distance from the anode (+) to the cathode (−). (For example in an IPG strip x = the strip length, for a standard slab gel, x = the width). z = the matrix depth.
	Physicochemical property range and distribution	As applicable, the details of the pH distribution of the matrix, including the overall pH range of the gel, if known. Details of the molecular weight distribution of matrix with appropriate measurement unit. Examples include linear pH 4-7, logarithmic apparent molecular mass 200-10 kDa.
	Acrylamide concentration	The acrylamide concentration of the gel, or each matrix. In the form of a single percentage (1%) or gradient (1-2%). For gradients include the gradient distribution if appropriate, (e.g. fixed, stepped or liner).
	Acrylamide:crosslinker ratio	The acrylamide to bisacrylamide ratio of the gel (Name of crosslinker and ratio described as "acrylamide:crosslinker")
	Gel lane	The number of lanes on the gel matrix
	Sample application	Description of the sample as applied to the matrix, giving: 1. Name of sample, Volume of loading buffer, Sample loaded per lane (if applicable for quantification, in SI measurement), and lane designation
	Buffer	Description of the running buffers used, in terms of name of buffer, components with concentrations.
	Equipment	Apparatus used for electrophoresis. Include brand and model.
	Electrophoresis conditions	The running conditions applied to the gel (To be given in terms of voltages versus time/kilovolt hours, (or appropriate measurements) and temperature).
3.1.2	**Staining**	Staining method used to develop the gel image.
3.1.3	**In gel digestion**	
	Digestion buffer	Buffer used for digestion, indicating components, concentration and pH
	Enzyme	Enzyme description (solubilization buffer and concentration).
	Incubation parameters	Incubation time and temperature
	Previous treatment	Previous treatment if required (e.g. Reduction and/or alkylation)
	Automatic digestor	If required, brand and model of the apparatus used
3.2	**HPLC and liquid digestion**	(From HUPO-PSI MIAPE::Column Chromatography vs 1.0, June 2008)
	Starting Material	Mass in SI units (cells or tissues) or volume of fluid submitted for separation.

3.2.1	**Equipment**	
3.2.1.1	**Product details for column**	
	Make	The name of the manufacturer
	Model	The model number provided by the manufacturer
	Separation mode	A description of the type of column being used (e.g., Separation mechanism: affinity, anion exchange, cation exchange, reverse phase, size).
3.2.1.2	**Physical characteristics of column**	
	Length	The length of the column.
	Diameter	The internal diameter of the column.
	Description of stationary phase	A description of the constituents of the stationary phase, including the name of the packing material and the particle size.
3.2.1.3	**Chromatography system used for separation, where applicable**	The name of the manufacturer and the model name provided by the manufacturer.
3.2.1.4	**Mobile phase: for each mobile phase**	
	Name of mobile phase	Name used to refer to mobile phase in Properties of Column run
	Description of the constituents	For each constituent, a description and the concentration
3.2.1.5	**Properties of the column run**	
	Time	The length of time for which the column is run. The value can be provided as a duration of time,
	Gradient	The proportion of each of the mobile phases at a point in time, or the function describing the gradient, including the overall duration of the gradient. There may be several steps that together make up the gradient.
	Flow rate	The rate at which the mobile phase is applied to the column, including the time period for which this holds if it varies during the experiment.
	Temperature	The temperature at which the column is run, including the period for which this holds if it varies during the experiment
	Separation purpose	Analytical or preparative
3.2.1.6	**Column outputs - (a) detection**	
	Equipment used for detection	Make, model and description
	Type	A description of the kind of detector (e.g. UV) and a description of control properties of the detector, such as the wavelength that is being detected
	Equipment settings	A description of control properties of the detector, such as the wavelength that is being detected
	Timescale over which data was collected	The time range covered by the trace produced by the detector
	Trace	The location and format of the trace
3.2.1.7	**Column outputs - (b) fractions (if required)**	
	Fraction name	An optional name, unique within a run, by which a fraction can be referenced.

	Fraction description	Either a description of the procedure by which the fractions were collected (i.e. start/end time, size (time or volume), mode (fixed or peak directed), or a description of the individual fractions (e.g. time of collection, volume)
3.2.2	**Liquid digestion of fractions, if required**	
	Digestion buffer	Buffer used for digestion, indicating components, concentration and pH
	Enzyme	Enzyme description and concentration
	Incubation parameters	Incubation time and temperature
	Previous treatment	Previous treatment if requires (e.g. Reduction and/or alkylation)
3.2.3	**Other parameters**	Other parameters performed not included in 3.2.1 to 3.2.2
4. Peptide Purification/Fractionation		
4.1	**HPLC**	(From HUPO-PSI MIAPE::Column Chromatography vs 1.0, June 2008)
	Origin of the sample	The origin of the peptide mixture submitted to purification: solution from in-gel digestion, liquid digestion, etc
4.1.1	**Equipment**	
4.1.1.1	**Product details for column**	
	Make	The name of the manufacturer
	Model	The model number provided by the manufacturer
	Separation mode	A description of the type of column being used (e.g., Separation mechanism: affinity, anion exchange, cation exchange, reverse phase, size).
4.1.1.2	**Physical characteristics of column**	
	Length	The length of the column.
	Diameter	The internal diameter of the column.
	Description of stationary phase	A description of the constituents of the stationary phase, including the name of the packing material and the particle size.
4.1.1.3	**Chromatography system used for separation**	The name of the manufacturer and the model name provided by the manufacturer.
4.1.2	**Mobile phase: for each mobile phase**	
	Name of mobile phase	Name used to refer to mobile phase in Properties of Column run
	Description of the constituents	For each constituent, a description and the concentration
4.1.3	**Properties of the column run**	
	Time	The length of time for which the column is run. The value can be provided as a duration of time,
	Gradient	The proportion of each of the mobile phases at a point in time, or the function describing the gradient, including the overall duration of the gradient. There may be several steps that together make up the gradient.

	Flow rate	The rate at which the mobile phase is applied to the column, including the time period for which this holds if it varies during the experiment.
	Temperature	The temperature at which the column is run, including the period for which this holds if it varies during the experiment
	Separation purpose	Analytical or preparative
4.1.4a	**Column outputs - (a) detection**	
	Equipment used for detection	Make, model and description
	Type	A description of the kind of detector (e.g. UV) and a description of control properties of the detector, such as the wavelength that is being detected
	Equipment settings	A description of control properties of the detector, such as the wavelength that is being detected
	Timescale over which data was collected	The time range covered by the trace produced by the detector
	Trace	The location and format of the trace
4.1.4b	**Column outputs - (b) fractions (if required)**	
	Fraction name	An optional name, unique within a run, by which a fraction can be referenced.
	Fraction description	Either a description of the procedure by which the fractions were collected (i.e. start/end time, size (time or volume), mode (fixed or peak directed), or a description of the individual fractions (e.g. time of collection, volume)
4.1.5	**Other parameters**	Other parameters not included in 4.1.1.
5. Phosphopeptide Enrichment (Repeat the steps as necessary)		
5.1	**Method 1**	
	Method description	Brief description of the method (e.g. IMAC purification)
5.1.1	**Starting sample**	
	Origin of the sample	The origin of the peptide mixture submitted to purification: solution from in-gel digestion, eluate from HPLC or other peptide enrichment procedure, etc
	Volume	Initial volume of the peptide mixture to be purified
	Pretreatment	Volume reduction, sample dilution, dessalting, etc...
	Loading buffer	Buffer (type and volume) used to dissolve or dilute the sample and to load it into the resin
5.1.2	**Affinity material**	
	Description of the affinity material	Material used for phosphopeptide enrichment. Include name of the manufacturer, reference and, if required, type of metal loaded in the material .
	Pretreatment of the affinity material	Procedures used to suspend, wash and conditionate the affinity material (buffers, time and volume).
5.1.3	**Affinity purification procedure**	
	Volume of sample	Volume of sample used for purification (in SI units).
	Amount of affinity material	Amount of affinity material used for purification (in SI units).

	Type of purification	In-batch or in-column purification (indicate the type of tube, holder or other container used for the purification).
	Incubation time	Incubation time of the sample with the resin
	Incubation temperature	Incubation temperature of the sample with the resin
	Washing buffer	Buffer (type and volume) used to wash the affinity material after incubation.
	Elution buffer	Buffer (type and volume) used to elute the phosphopeptides from the resin.
	Name of the obtained fractions	Code identifying the eluted extract and/or. if appropiate, the unretained fraction to be submitted to other analyses.
5.1.4	**Other parameters**	Other parameters not included in 5.1.1 to 5.1.3.