Phosphopeptide Standard Mixture (P33357)

Quick Facts

Storage upon receipt:

- ≤–20°C

Introduction

The phosphopeptide standard mixture is formulated as a standard for MALDI-MS. This mixture contains three unphosphorylated peptides and four phosphopeptides in equimolar amounts. Phosphoserine (pS), phosphothreonine (pT), and phosphotyrosine (pY) monophosphopeptides and a peptide containing both pT and pY are represented (Table 1, Figure 1).

Contents

Phosphopeptide standard mixture, 400 pmol of each peptide (see Table 1)

Preparation of Standards

1.1 Dissolve the phosphopeptide standard mixture to the desired concentration in purified water. Use as an internal or external standard for MS analysis. Two MALDI spotting techniques are described below. For MALDI, apply a spot containing 1 pmol or less of each peptide, depending on the sensitivity of the instrument.

Table 1. Peptide standards (400 pmol each).

Peptide	M+1	Amino Acid Sequence
Angiotensin II	1,046.54	DRVYIHPF
Angiotensin I	1,296.69	DRVYIHPFHL
Myelin basic protein, fragment 104–118	1,578.85	GKGRGLSLSRFSWGA
pTpY peptide (MAP kinase, fragment 177–189)	1,669.67	DHTGFLpTEpYVATR
pY peptide (insulin receptor, fragment 1142–1153)	1,702.75	TRDIpYETDYYRK
pT peptide	1,720.89	VPIPGRFDRRVpTVE
pS peptide (RII phosphopeptide, fragment 81–99)	2,192.09	DLDVPIPGRFDRRVpSVAAE

1.2 Store the phosphopeptide standard mixture at ≤–20°C. Prolonged storage at room temperature will result in dephosphorylation of the phosphotyrosine residues.

MALDI Spotting

Dried-Droplet Method

Spot 0.5–1 µL of a sample onto a MALDI target plate. Spot an equal volume of 5–10 mg/mL α-cyano-4-hydroxycinnamic acid (CHCA) dissolved in 50% (v/v) acetonitrile, 0.1% (v/v) trifluoroacetic acid on top of the sample, and allow it to air-dry. This simple and fast method is recommended for samples containing at least 0.5 pmol of phosphopeptide.

Nitrocellulose Method

Prepare 40 mg/mL CHCA in acetone, and 20 mg/mL nitrocellulose in acetone. Vortex both solutions for 5–10 minutes, then make a matrix solution by mixing 200 µL of the CHCA solution

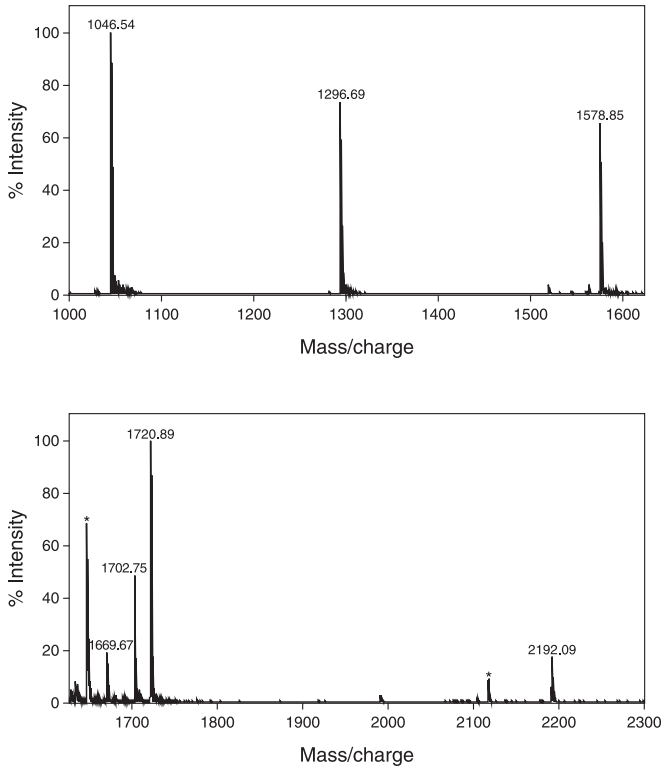

Figure 1. A spot of the peptide standard mixture containing 1 pmol of each peptide was applied to a MALDI plate and analyzed in positive reflectron mode. Peaks marked with * indicate in-flight dephosphorylation of pT and pS peptides, showing up as a loss of 74 daltons.

with 100 µL of the nitrocellulose solution and 100 µL of isopropanol. Mix 1.5 µL of a sample with an equal volume of the matrix solution, and deposit 1.5 µL of the mixture onto the MALDI target plate. Allow the spot to dry at ambient temperature, then place 5 µL of 5% formic acid on top of the spot for 10 seconds and remove by reverse pipetting. Allow the spot to dry. Repeat the 10 second wash with deionized water, and allow the spot to dry. This method is reported to markedly enhance peptide detection for samples with <1 pmol of peptide. It also enables rinsing of salts from the sample directly on the MALDI plate.[1]

General Notes for MALDI-MS Sample Preparation

Salt Removal

Salt concentrations above 10 mM generally interfere with MALDI analysis. Desalting is labor intensive but allows for maximum recovery of most peptides in a small volume free of interfering salts. If the analyte concentration is sufficient, an experimental sample may be diluted to reduce the salt concentration to acceptable levels prior to spotting onto the MALDI plate. Otherwise, desalt samples with reversed-phase cleanup devices, e.g., ZipTip® pipette tips (Millipore), Vivapure® C18 micro spin columns (Vivascience), or equivalent, according to manufacturer's instructions. Samples must be acidified to ≤pH 4 with glacial acetic acid prior to reversed-phase desalting/concentration and may be eluted with 50% acetonitrile, 0.1% trifluoroacetic acid directly onto the plate if maximum recovery is required. One drawback of reversed-phase desalting is that very hydrophilic peptides may not be recovered. If >50% acetonitrile is required for elution, dilute the sample after elution so that the acetonitrile concentration is ≤50% prior to spotting onto the plate. Higher acetonitrile concentrations may adversely affect crystal formation.

Phosphopeptide Detection

Phosphopeptide detection may be enhanced by addition of 25–50 mM ammonium salts (e.g., ammonium sulfate, ammonium citrate, or ammonium acetate), but the degree of enhancement depends on the peptide. Techniques include mixing the ammonium solution with the sample or matrix solution at a 1:1 (v/v) ratio, or spotting an equal volume of the ammonium solution on top of the sample on the target plate. Some phosphopeptide signals may be enhanced relative to other peaks by analysis in negative-ion mode.

Reference

1. Anal Biochem 279, 1 (2000).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
P33357	phosphopeptide standard mixture *400 pmol of each peptide*	2800 pmol
C33355	Captivate™ Microscale Phosphopeptide Isolation Kit *for magnetic separation* *50 isolations of 10 µmol each*	1 kit
E33202	EZQ® Phosphopeptide Quantitation Kit *1000 assays*	1 kit
P33203	Pro-Q® Diamond LC Phosphopeptide Detection Kit	1 kit
P33706	Pro-Q® Diamond Phosphoprotein/Phosphopeptide Microarray Stain Kit	1 kit

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