Iodoacetamido-LC-Phosphonic Acid (6C-CysPAT), Single Use

Catalog Numbers A52285

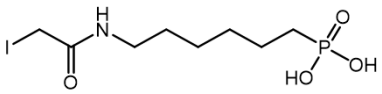
Doc. Part No. 2162754 Pub. No. MAN0025825 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Iodoacetamido-LC-Phosphonic Acid (6C-CysPAT) is a sulfhydryl-reactive alkylating reagent that contains a phosphonic acid group for enrichment of cysteine-containing peptides. In contrast to phosphate groups, the phosphonic acid group of 6C-CysPAT is not a substrate for phosphatases, which can be used to remove endogenous phosphate-modified peptides before or after enrichment. Alkylation of reduced cysteine-containing proteins/peptides with 6C-CysPAT results in a covalent thioester bond with a modification mass of 221.082 Da. After protein alkylation and enzymatic digestion, labeled peptides can be enriched using immobilized metal affinity chromatography (IMAC) or metal oxide affinity chromatography (MOAC).

Contents and storage

Contents	Cat. No.	Amount	Storage
Iodoacetamido-LC-Phosphonic Acid (6C-CysPAT) MW = 348.99 	A52285	10 × 3.5 mg	Store at 4°C protected from light.

Additional information

- Iodoacetamido-LC-Phosphonic Acid (6C-CysPAT) is unstable and light-sensitive. Prepare solutions immediately before use and perform alkylation in the dark. If 6C-CysPAT is present in limiting quantities and a slightly alkaline pH, cysteine modification will be the exclusive reaction. Excess 6C-CysPAT or non-buffered reagent can also alkylate primary amines (lysine, N-termini), thioethers (methionine), imidazoles (histidine) and carboxylates (aspartate, glutamate).
- Excess, unreacted Iodoacetamido-LC-Phosphonic Acid (6C-CysPAT) must be removed before enrichment of labeled peptides. Methanol/chloroform precipitation is recommended for protein-level clean up. The peptide clean-up columns in the EasyPep™ Mini MS sample prep kits are recommended for peptide-level clean up. C18 and peptide desalting columns are not recommended for 6C-CysPAT clean up.

Required materials not supplied

- 100 mM TEAB solution (Cat. No. 90114, diluted 1:10 with ultrapure water)
- EasyPep™ Mini MS Sample Prep Kit (Cat. No. A40006)
- Phosphopeptide enrichment kit (Cat. No. A32993, A32992, A52283, or A52284)

Reduce and alkylate proteins

This protocol is designed to reduce and alkylate proteins using EasyPep™ Mini MS Sample Prep Kit peptide clean-up columns for removing excess reagent.

1. Prepare 50–100 µg of protein extract in 100 µL of EasyPep™ Lysis Buffer (or 100mM TEAB).
2. Add 50 µL of Reduction Solution (i.e., 20mM TCEP) to the protein in lysis solution.
3. Prepare a 20 mM stock solution by dissolving 3.5 mg of Iodoacetamido-LC-Phosphonic Acid (6C-CysPAT) using 500 µL of 100 mM TEAB buffer.
4. Add 50 µL of 20 mM 6C-CysPAT solution to the protein for a final concentration of 5 mM (e.g., equimolar to reducing agent concentration).

   Note: It may be necessary to titrate the final 6C-CysPAT concentration to determine the optimal molar excess for protein labeling. Typically, using an equimolar concentration of 6C-CysPAT to reducing agent (1:1, 6C-CysPAT:TCEP) is sufficient to observe complete labeling of reduced cysteines with minimal off-target labeling, but more reagent may be necessary to label some protein complexes. Higher molar excess of 6C-CysPAT may require protein level clean up before digestion as trypsin activity may be inhibited.
5. Incubate samples at 95°C for 5 minutes and then cool to room temperature.
6. Proceed with sample digestion and peptide clean up as described using the EasyPep™ Mini MS Sample Prep Kit.

   Note: The EasyPep™ protocol is compatible with Iodoacetamido-LC-Phosphonic Acid (6C-CysPAT) at the concentrations mentioned above. If using an alternative sample prep procedure or higher molar excess of C6-CysPAT, methanol/chloroform precipitation should be performed prior to digestion.

Enrich labeled peptides

This protocol is designed to enrich Iodoacetamido-LC-Phosphonic Acid (6C-CysPAT)-labeled peptides using the High-Select™ Fe-NTA Magnetic Phosphopeptide Enrichment Kit (Cat. No. A52283). Lyophilize clean peptide samples using a speedvac before enrichment.

1. Dissolve 50–100 µg of clean, labeled peptide using 100 µL of Binding/Wash Buffer in a low protein-binding tube.
2. Transfer 10 µL of the magnetic Fe-NTA bead slurry to a new microfuge tube and remove the storage buffer using a magnetic stand.
3. Wash the resin by adding 20 µL of Binding/Wash Buffer, then vortex briefly and remove the buffer using a magnetic stand.
4. Repeat the wash once for a total of 2 washes.
5. Add 10 µL of the Binding/Wash Buffer to the resin and transfer slurry to the peptide solution (1:10, µL bead slurry:µg sample).
6. Incubate at room temperature for 30 minutes with end-over-end mixing.
7. Collect the beads using a magnetic stand and remove the unbound peptide solution.
8. Wash the resin by adding 20 µL of Binding/Wash Buffer, then vortex briefly and remove the buffer using a magnetic stand.
9. Repeat the wash twice for a total of three washes.
10. Wash the resin by adding using 20 µL of MS-grade water, then vortex briefly and remove the water using a magnetic stand.
11. Add 20 µL of Phosphopeptide Elution Buffer, then vortex briefly and incubate for 1 minute at room temperature.
12. Repeat elution for a total of 2 elutions.
13. Combine the eluted phosphopeptide samples, transfer to a low protein-binding tube, and speedvac dry before LC-MS analysis. We recommend centrifugation of the eluted peptide samples at 10,000 × *g* for 1 min before transferring supernatant to avoid bead carryover before drying. Use of C18 tips or trap columns are recommended to ensure resin particles do not interfere with LC-MS analysis.
14. Resuspend dried phosphopeptide sample using 0.1% FA for LC-MS analysis.

Related products

Product	Cat. no.
Iodoacetamide, single-use	A39721
Pierce™ Quantitative Colorimetric Peptide Assay Kit	23275
Pierce™ Quantitative Fluorometric Peptide Assay	23290
Pierce™ CIP Alkaline Phosphatase	31391
High-Select™ TiO_2 Phosphopeptide Enrichment Kit	A32993
High-Select™ Fe-NTA Phosphopeptide Enrichment Kit	A32992
High-Select™ Fe-NTA Magnetic Phosphopeptide Enrichment Kit	A52283
High-Select™ Fe-NTA Magnetic Agarose	A52284

Troubleshooting

Observation	Possible cause	Recommended action
Minimal or no cysteine modification observed	Disulfide bonds not reduced.	Use appropriate concentration of reducing agent (TCEP, DTT) to reduce disulfide bonds.
	Used inappropriate conjugation buffer.	Avoid buffers that contain sulfhydryls or that are not at a slightly alkaline pH.
Poor enrichment of modified peptides	Incomplete removal of excess reagent.	Clean up protein samples using methanol/chloroform precipitation or peptide samples using peptide clean up columns from EasyPep™ Mini MS Sample Prep Kit.
	Off-target labeling of amines.	Reduce the amount of reagent, reaction temperature or incubation time.
	Insufficient capacity of phospho-enrichment resin.	Use more resin (Fe-NTA agarose, TiO2) for enrichment or split sample to enrich over multiple columns.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
A.0	18 November 2021	New manual for Iodoacetamido-LC-Phosphonic Acid (6C-CysPAT), Single Use.