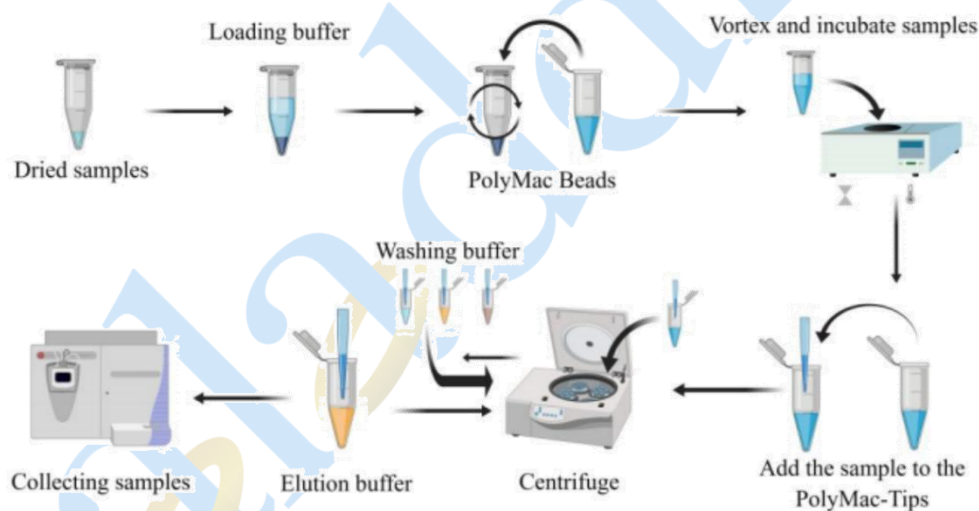


Phosphopeptide Protein (Peptide Fragment) Extraction Kit

P1456460

Introduction:

Due to the low stoichiometry of phosphorylation, the concentration of phosphopeptides is a key step for the successful implementation of phosphoproteomics experiments. PolyMAC provides an effective and significantly improved method to achieve more complete enrichment of phosphopeptides. This highly selective enrichment method can be used for most complex samples because it offers optimal specificity and recovery rate. PolyMAC is a polymer-based immobilized metal ion affinity capture method with excellent phosphoproteome coverage and recovery rate. It is a soluble nanopolymer designed to interact with phosphopeptides in solution, and then be captured on a solid-phase carrier for washing and phosphopeptide elution. Compared with the commonly used titanium dioxide and immobilized metal ion affinity capture methods, PolyMAC has better reproducibility and enrichment of phosphorylated peptides, with the selectivity of phosphorylated peptides close to 95% and the recovery rate > 90%. Importantly, PolyMAC has very good recovery rate and selectivity for samples with low total protein phosphorylation levels.



Experimental Flowchart of Phosphopeptide Protein (Peptide Fragment) Extraction Kit (P1456460).

Product Components and Storage Conditions:

P1456460	Component	24T	48T	96T	Storage
P1456460A	PolyMAC Beads	0.75 mL	1.5 mL	3 mL	4°C
P1456460B	PolyMAC Loading Buffer	25 mL	50 mL	100 mL	RT
P1456460C	PolyMAC Washing Buffer I	25 mL	50 mL	100 mL	RT
P1456460D	PolyMAC Washing Buffer II	25 mL	50 mL	100 mL	RT
P1456460E	PolyMAC Elution Buffer	3.75 mL	7.5 mL	15 mL	RT
P1456460F	PolyMAC Tips	24T	48T	96T	RT

Product Features:

Easy to operate: Phosphopeptides can be prepared quickly, requiring only a metal bath and a conventional centrifuge.

High stability: Strict quality inspection is conducted for each batch, ensuring high reproducibility of experimental results.

Operating Procedure:

1. Add 200 μ L of Loading Buffer to the dried sample (50 μ g - 100 μ g) after enzymatic hydrolysis and desalting, and fully resuspend the sample.
2. Vortex the PolyMAC Beads thoroughly (for 10 - 20 seconds), then take 25 μ L into a 1.5 mL centrifuge tube, centrifuge instantaneously for 2 - 3 seconds, and then remove the upper storage solution (The Beads settle quickly, so the operation should be fast when taking them) .
3. Add the resuspended sample to the centrifuge tube containing PolyMAC Beads, then place it on a mixer, and shake vigorously at 26°C, >1200 rpm for 25 minutes.
4. Add the sample to PolyMAC - Tips, first centrifuge at 20 g for 2 minutes, then centrifuge at 100 g for 1 minute to ensure that the sample solution flows out from the tip of the Tips into the centrifuge tube (The rotation speed should not be too high, and the operation should be fast) .
5. Wash once with 200 μ L of Loading Buffer: first centrifuge at 20 g for 2 minutes, then centrifuge at 100 g for 1 minute, and discard the filtrate. Repeat this step 3 times (The rotation speed should not be too high, and the operation should be fast) .
6. Wash once with 200 μ L of Washing Buffer I: first centrifuge at 20 g for 2 minutes, then centrifuge at 100 g for 1 minute, and discard the filtrate. Repeat this step 3 times (The

rotation speed should not be too high, and the operation should be fast) .

7. Wash once with 200 μ L of Washing Buffer II: first centrifuge at 20 g for 2 minutes, then centrifuge at 100 g for 1 minute, and discard the filtrate. Repeat this step 3 times (The rotation speed should not be too high, and the operation should be fast.)
8. Put the Tips into a new centrifuge tube to collect the eluted phosphopeptides. Add 50 μ L of Elution Buffer to the Tips, centrifuge at 20 g for 2 minutes, then add another 50 μ L of Elution Buffer, first centrifuge at 20 g for 2 minutes, then centrifuge at 100 g for 1 minute, and collect the filtrate (The rotation speed should not be too high, and the operation should be fast.)

Note: In steps 4 - 8, if the liquid flows down slowly, the rotation speed can be increased appropriately, gradually increasing to 50 g, and do not increase too much at one time!

9. If there is any solution remaining in the pipette tip, push it out into the collection tube with a pipette, lyophilize it, and store it at -80°C for mass spectrometry detection.

Precautions:

This product is only for scientific research use by professionals, and shall not be used for clinical diagnosis or treatment, nor for food or drugs.