

PHOSPHATASE REACTION FOR SIMPLE WESTERN CHARGE ASSAYS



INTRODUCTION

The phosphorylated peaks of a target protein in NanoPro™ 1000 and Peggy Sue™ Simple Western™ Charge assays can be identified by treating lysates with phosphatase and comparing the electropherograms to an untreated control. Peaks that shift as a result of phosphatase treatment indicate the phosphorylation state of a target protein. Here, we provide a protocol that uses lambda phosphatase, a Mn²⁺-dependent protein phosphatase that releases phosphate groups from phosphorylated serine, threonine, tyrosine and histidine residues. This method is based on the protocol provided in the Lambda Phosphatase Kit from New England Biolabs (NEB, PN P0753). Sources of phosphatase not described here may require further optimization for use in Simple Western Charge assays.

LAMBDA PHOSPHATASE REACTION PROTOCOL

MATERIALS

Lambda Phosphatase Kit (NEB, PN P0753) containing:

- 1 vial of 10X Protein MetalloPhosphatases (PMP) buffer
- 1 vial of 10 mM MnCl₂
- 1 vial of Lambda Protein Phosphatase

REACTION SETUP

1. Dilute lysate to 0.36 mg/mL in Bicine/CHAPS (ProteinSimple, PN 040-764) or Sample Diluent (ProteinSimple, PN 040-649).
2. Then mix the reaction as follows:
 - 1 µL 10X PMP buffer
 - 1 µL 10 mM MnCl₂
 - 1 µL Lambda Protein Phosphatase
 - 7 µL lysate

3. Incubate at 30 °C for 60 minutes.
4. Place the reaction mix on ice.
5. Prepare samples as usual for the NanoPro 1000 or Peggy Sue Simple Western Charge assays.

NOTES: For a negative control, incubate the lysate with the 10X PMP buffer and 10 mM MnCl₂ but no phosphatase enzyme. You can check for cross reactivity of lambda phosphatase with antibodies by running a capillary with no lysate but containing the lysate diluent (Bicine/CHAPS or Sample Diluent) and other reagents (PMP buffer, MnCl₂ and lambda phosphatase). Then, probe with antibodies.

We also tested the Lambda Protein Phosphatase from Sigma (PN P9614) and the Lambda Protein Phosphatase from EMD Millipore (PN 539514). These reagents can be used instead of the Lambda Phosphatase Kit (NEB, PN P0753) according to their manufacturer's specifications.