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Endo H/ PNGase F Assay for JESS Automated Western Blot

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol details an optimized Endo H and PNGase F digestion assay that produces samples that can be run with the JESS Automated Western Blot System.

Materials

10X Glycoprotein Denaturing Buffer (NEB, Catalog# B0701S)
10X GlycoBuffer 3 (NEB, Catalog# B0701S)
10X GlycoBuffer 2 (NEB, Catalog# B0701S)
10% NP-40 (NEB, Catalog# B0701S)
EndoH (NEB, Catalog# P0703S)
PNGase F (NEB, Catalog# P0704S)

10K Amicon Ultra Centrifugal Filter (Millipore, UFC5010BK)

Glycoprotein Denaturation

1 In an eppendorf tube, add 40 µg protein, 2 µL 10X Glycoprotein Denaturing Buffer, and water for a total reaction volume of 20 µL. Mix well.

2 Denature glycoprotein by heating reaction at 100 °C for 00:10:00. 10m

3 Split reaction volume into 2 eppendorf tubes with 10 uL in each tube. One tube will be used for the EndoH reaction, while the other will be used for the PNGaseF reaction.

Endo H

4 Add the following to one reaction tube from step 3: 2 µL 10X GlycoBuffer 3, 2 µL Endo H, 6 µL water. Total volume will be 20 µL.

5 Mix gently.

6 Incubate reaction at 37 °C for 01:00:00 1h

PNGase F

7 Add the following to the second reaction tube from step 3: 2 µL 10X GlycoBuffer 2, 2 µL NP-40, 6 µL water, 1 µL PNGaseF. Total volume will be 21 µL.

8 Mix gently.

9 Incubate reaction at 37 °C for 01:00:00. 1h

Desalting and Washing for JESS Automated Western Blot System (Bio-Techne)

preparation

10 Add 480 µL and 479 µL of 0.1X Sample Buffer (Bio-Techne) to the Endo H and PNGaseF reaction tubes respectively to make the final volume 500 µL .

11 Add all 500 µL of each reaction to a 10K Amicon Ultra Centrifugal Filter

12 Spin at 14000 x g for 00:20:00 . You will recover a final volume of 20 µL , and the protein concentration recovered will be ~ [M] 1 mg/mL . 20m

JESS Sample Preparation 5m

13 Add 4 parts of the washed Endo H/ PNGaseF sample from step 12 with 1 part of 5X Fluorescent Master Mix (Bio-Techne).

14 Vortex to mix

15 Incubate sample at 95 °C for 00:05:00 . 5m

16 Load into JESS microplate (Bio-techne) to be detected by the Automated Western Blot System.

Protocol references

https://www.neb.com/en-us/protocols/2012/10/18/endo-hf-protocol
https://www.neb.com/en-us/protocols/2014/07/31/pngase-f-protocol