Nov 27, 2025

Preparation method of pyridylaminated *N*-glycans

PLOS One

DOI
https://dx.doi.org/10.17504/protocols.io.n2bvjez1pgk5/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.n2bvjez1pgk5/v1

External link: https://doi.org/10.1371/journal.pone.0336565

Protocol Citation: Riko Makino, Shunji Natsuka 2025. Preparation method of pyridylaminated N-glycans. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvjez1pgk5/v1

Manuscript citation:
Makino R, Natsuka S (2025) Optimizing the preparation of labeled N-glycans for rapid, simplified, and high-precision analysis. PLOS One 20(12). doi: 10.1371/journal.pone.0336565

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

Created: September 30, 2025

Last Modified: November 27, 2025

Protocol Integer ID: 228515

Keywords: N-glycan, human serum, human urine, cultured cells, glycan, glycans this protocol, preparation of human serum, preparation method, purification, blotglyco, aminopyridine, protein sample, fluorescent labeling, preparation

Abstract

This protocol describes the purification of *N*-glycans from approximately 50 µg of protein samples, followed by fluorescent labeling with 2-aminopyridine. The procedure utilizes Rapid PNGase F (New England BioLabs) and BlotGlyco (Sumitomo Bakelite). Representative examples include the preparation of human serum, human urine, and CHO-K1 membrane fractions; however, the protocol is also applicable to other types of samples.

Protocol materials

BlotGlyco **Sumitomo Bakelite Catalog #**BS-45414

Rapid PNGase F **New England Biolabs Catalog #**P0710S

Sample preparation 1: for glycoprotein

1m

1 50-100 µg glycoprotein (dry sample) in 0.2 mL PCR tube

2 add 15 µL of DDW 30s

3 vortex 30s

Sample preparation 2: for human serum

1m

4 1 µL of human serum in 0.2 mL PCR tube

5 add 15 µL of DDW 30s

6 vortex 30s

Sample preparation 3: for human urinary protein

1h

7 50 µg of human urinary protein ppt. (dry sample) in 0.2 mL PCR tube

8 add 16 µL of DDW 30m

9 vortex 30m

Sample preparation 4: for CHO-K1 cell membrane fraction

2m

10 1 mg of CHO-K1 cell membrane fraction (dry sample) in 0.2 mL PCR tube

11 add XX µL of 0.1% NP-40 (10 µL of 10% NP-40, 990 µL of DDW) 1m

12 vortex 30s

13 transfer 16 µL (protein content 50-100 µg) to 0.2 mL PCR tube 30s

Enzymatic dygestion using Rapid PNGase F 13m 30s

14 add 4 µL of Rapid PNGase F Buffer (5x) to sample solution, mix gently with a pipette —total reaction volume to 20 µL 30s

Rapid PNGase F **New England Biolabs** **Catalog #**P0710S

15 denature at 80 °C for 00:02:00 2m

16 cool down the sample solution 30s

17 add 1 µL of Rapid PNGase F, mix by pipetting 30s

18 incubate at 50 °C for 00:10:00 10m

Glycan purification and fluorescent labeling using BlotGlyco 8h 59m 10s

19 50 µL of polymer beads suspension in the reaction tube 30s

BlotGlyco **Sumitomo Bakelite** **Catalog #**BS-45414

20 wash polymer beads with 100 µL of DDW, twice, spin down to remove water completely 1m

Note

Modification from manufacturer's protocol: wash polymer beads with 100 µL of DDW, twice

21 replace the reaction tube into a new 1.5 mL tube 10s

22 add 180 µL of 2% acetic acid in acetonitrile to the reaction tub 20 µL 1m
(20 µL of 980 µL 980 µL 980 µL 980 µL tic 980 µL ci 980 µL of acetonitrile)

23 add sample solution to the reaction tube, mix by pipetting 30s

Note

Modification from manufacturer's protocol: mix by pipetting.

24 heat at 80 °C for 01:00:00 , without cover (the solvent has completely evaporated.) 1h
(If the beads are not dry completely, continue heating for another 00:15:00)

25 replace the reaction tube into the 2 mL tube 30s

26 wash with 200 µL of 2M guanidine solution 1m
(1.9 g guanidine hydrochloride, 10 mL of DDW)

27 wash with 200 µL of DDW, mix by pipetting 1m

Note

Modification from manufacturer's protocol: mix by pipetting.

28 wash with 200 µL of DDW 1m

29 wash with 200 µL of 1% triethylamine in MeOH, mix by pipetting (100 µL of triethylamine, 9.9 mL of MeOH) 1m

Note

Modification from manufacturer's protocol: mix by pipetting.

30 wash with 200 µL of 1% triethylamine in MeOH 1m

31 replace the reaction tube into the 1.5 mL tube 10s

32 add 100 µL of 1% triethylamine in MeOH, mix by pipetting (15 µL of acetic anhydride, 135 µL of MeOH) 1m

Note

Modification from manufacturer's protocol: mix by pipetting.

33 leave at room temperature for 00:30:00 30m

34 replace the reaction tube into the 2 mL tube 10s

35 spin down to remove the fluid 30s

36 wash with 200 µL of DDW, twice 2m

37 replace the reaction tube into the 1.5 mL tube 10s

38 add 20 µL of DDW 30s

39 add 180 µL of 2% acetic acid in acetonitrile, mix by pipetting 1m
(100 µL of acetic acid, 4.9 mL of acetonitrile)

Note

Modification from manufacturer's protocol: mix by pipetting.

40 heat at 70 °C for 01:30:00 , without cover (the solvent has completely evaporated.) 1h 30m
(If the beads are not dry completely, continue heating for another 00:15:00

41 add 30 µL PAtion reagent 30s
(138 mg of 2-aminopyridine, 50 µL of acetic acid)

42 heat at 80 °C for 01:00:00 1h

43 add 110 µL of reduction reagent , mix by pipetting 30s
(100 mg of Me_2NH-borane, 40 µL of acetic acidtic acid, 25 µL of DDW)

Note

Modification from manufacturer's protocol: mix by pipetting.

44 heat at 80°C for 35 min 35m

45 replace the reaction tube into a new 1.5 mL tube 10s

46 13.200 rpm, 25°C, 00:01:00 1m 30s

47 add 1080 µL of acetonitrile to prepare 90% acetonitrile sample aliquot 1m

Note

Modification from manufacturer's protocol: prepare 90% acetonitrile sample aliquot, considering the volatility of acetic acid.

48 add the sample aliquot to the cleanup column in a centrifuge 10s

49 washed with 200 µL of DDW 1m

50 washed with 200 µL of acetonitrile, twice 2m

51 wlet the fluid drop until completely loaded 20m

Note

Modification from manufacturer's protocol: wait until all the liquid has been loaded onto the column.

52 2000 rpm, 25°C, 00:01:00 and 13200 rpm, 25°C, 00:00:30 2m

Note

Modification from manufacturer's protocol: apply the solution to the column by gentle centrifugation, then spin briefly at high speed to pass the liquid through. Perform the following centrifugation steps in the same manner.

53 wash with 600 µL of 95%(v/v) acetonitrile, cfg. 2000 rpm, 25°C, 00:01:00 and 13200 rpm, 25°C, 00:00:30 2m
(1.9 mL of CH_3CN, 100 µL of DDW)

54 wash with 600 µL of 95%(v/v) acetonitrile, 2000 rpm, 25°C, 00:01:00 and 13200 rpm, 25°C, 00:00:30 2m

55 replace the reaction tube into a new 1.5 mL tube 10s

56 add 25 µL of DDW 30s

57 2000 rpm, 25°C, 00:01:00 and 13200 rpm, 25°C, 00:00:30

58 add 25 µL of 0.1% acetic acid 30s
(1% acetic acid: 10 µL of 100% acetic acid, 990 µL of DDW)
(0.1% acetic acid: 10 µL of 1% acetic acid, 90 µL of DDW)

59 2000 rpm, 25°C, 00:01:00 and 13200 rpm, 25°C, 00:00:30 2m

60 transfer collected solution to 0.6 mL PCR tube with 200 µL of 0.28% ammonium aq. 2m
(2.8% ammonium aq.: 200 µL of 28% ammonium aq., 1.8 mL of DDW)
(0.28% ammonium aq.: 100 µL of 2.8% ammonium aq., 900 µL of DDW)

Note

A step to remove *O*-acetylated by-products

61 heat at 70 °C for 00:30:00 30m

62 lyophilize 3h

63 dissolved in 100 µL of DDW 1m

64 RP-LC/MS analysis

Note

LC/MS condition:
Hanzawa K, Suzuki N, Natsuka S. Structures and developmental alterations of N-glycans of zebrafish embryos. *Glycobiology*, 2017;27(3):228–245. DOI: 10.1093/glycob/cww124