ProMTag Multiomics DNA, RNA, Protein (ProMTag Multi) Sample Preparation Kit Guide

For extraction and cleanup of genomic DNA, total RNA, and protein and digestion of extracted protein

Protocol optimized for up to 100 µg protein with concentrations 1 mg/mL or greater

Contents

Kit component	Quantity	Storage
ProMTag	40 µL	-20°C
MT-Trypsin	225 µL	-20°C
Lysis buffer (LB)	10 mL	4°C
NA precipitation buffer (NAP)	2 mL	4°C
NA elution buffer 1 (NAE1)	600 µL	4°C
NA elution buffer 2 (NAE2)	1 mL	4°C
Wash buffer NA (WBN)	8 mL	4°C
Wash buffer 1 (WB1)	8 mL	4°C
Wash buffer 2 (WB2)	8 mL	4°C
Protein elution buffer (EB)	1 mL	4°C
ProMTag capture resin	900 µL	4°C
IAA	8 x 20 µL	4°C
0.5 mL ProMTag Resin Capture (RC) tubes	8 tubes	4°C
2 mL Waste collection (adapter) tubes	8 tubes	4°C
1.5 mL Nucleic acid collection tubes	16 tubes	4°C
1.5 mL Low protein binding tubes	16 tubes	4°C

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Storage

Store the entire kit at 4°C, EXCEPT: ProMTag and MT-Trypsin, store at -20°C. We recommend using your kit within 6 months of receiving it.

Safety

Always protect yourself appropriately when working with chemicals. This includes, but is not limited to, utilizing an appropriate lab coat, disposable gloves, and protective eye goggles. For more information, please consult the appropriate Safety Data Sheets. These are available online at https://www.impactproteomics.com/resources.

The ProMTag, NAP, WBN, WB1, and WB2 contain various amounts of acetonitrile. Please dispose of these appropriately and avoid open flames.

LB contains guanidine thiocyanate. Avoid contact with skin and eyes. If skin contact occurs, remove contaminated garments and rinse skin thoroughly with water. If eye contact occurs, remove contact lenses if applicable and rinse with plenty of water. If eye contact occurs, immediately call a poison center or doctor. Guanidine thiocyanate is harmful to aquatic life, ensure proper disposal. Avoid combining LB with bleach or acids.

EB contains formic acid. Avoid contact with skin and eyes. If skin contact occurs, remove contaminated garments and rinse skin thoroughly with water. If eye contact occurs, remove contact lenses if applicable and rinse with plenty of water.

Equipment and reagents you will need before you start:

- Protein sample, cell lysate, or protein source
- Pipettes and pipette tips
- Benchtop centrifuge (mini or full size)
- Sample rotator (rotisserie or carousel)
- Heating block
- Vortex
- Ultrapure, nuclease-free deionized water

- DNase and RNase enzymes if DNase and/or RNase treatment is necessary for intended downstream applications
- 2-Mercaptoethanol
- RNase-free pestle homogenizer if working with a tissue sample
- RNase inhibitor (optional)

Cell lysis and preparation of the biological sample for ProMTag Multi processing

The ProMTag Multi kit is intended to prepare mass spectrometry-ready protein and sequencing-ready DNA and RNA. For the best results, cell lysis must be as thorough as possible. Furthermore, lysis must be gentle enough to avoid damage or degradation of the DNA and RNA. Avoid sonicating or freezing and thawing samples prior to ProMTag Multi processing when possible.

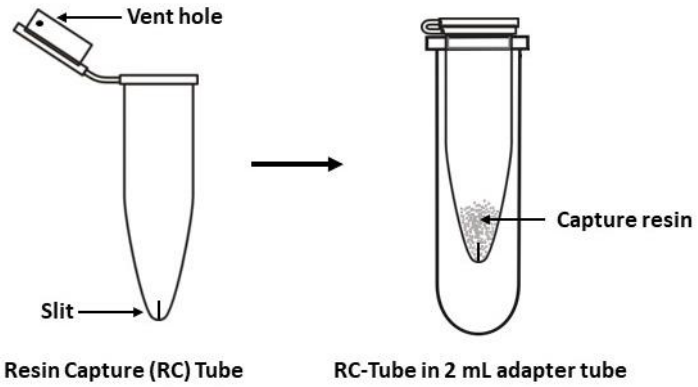
While we recommend utilizing the lysis buffer included with this kit, other lysis buffers are compatible with the kit. You can use your own lysis buffer **as long as it does not contain TRIS (or any other buffer with primary amines) and is ~pH 8.0**. If your lysis technique uses TRIS, we recommend switching to 100 mM HEPES pH 8.0. If you need advice on lysis for your particular sample, we are available to help. Simply email us at info@impactproteomics.com.

If your lysis buffer does not contain a strong denaturant, we **highly** recommend using a protease inhibitor in your lysis buffer to prevent protein degradation. We also highly recommend utilizing a lysis buffer containing a high concentration of guanidine thiocyanate to help maintain RNA integrity.

The provided lysis buffer must be supplemented with 2-Mercaptoethanol (not included) to a final concentration of 284 mM immediately prior to use. If your sample is RNase-rich, we recommend supplementing NAE1 with RNase inhibitor immediately before the first nucleic acid elution step.

Other notes to consider before you begin

- The RC-tubes have two features that distinguish them from typical spin columns: a fine slit to retain resin instead of a frit, and a hole in the rim of the cap to prevent loss of liquid when closing the tubes.



- Avoid touching the bottom of the RC-tubes. Keep the RC-tube in a waste or collection tube, or a low protein binding tube when incubating or vortexing, except when mixing on a rotisserie.
    a. We recommend briefly vortexing or tapping the RC-tubes at multiple points throughout the protocol to aid in resuspension of the resin. **Never vortex the RC-tube alone.** Always vortex by placing the RC-tube in a 1.5 mL or 2 mL tube as an adapter to avoid touching the bottom of the RC-tube.
- We do not recommend pipetting to mix at any stage where the capture resin is present, as the resin will stick to the tip and result in suboptimal yield.
- All centrifugation steps may be performed on a benchtop centrifuge at room temperature.
- For best results, keep the resin suspended during all incubation steps. This can be done using a 360° rotisserie (recommended) or a carousel. We do not recommend shaking to keep the resin suspended, but if you do ensure you use **gentle** agitation.
- To avoid nucleic acid degradation, ensure all pipette tips are nuclease free. Also, keep samples at 4°C or on ice as much as possible until nucleic acid elution is completed.
- The protocol takes 6-8 hours to perform, depending on the number of samples being processed and the sample type being prepared.

Protocol for DNA, RNA, and protein sample extraction, cleanup, and protein digestion using the ProMTag Multi kit

Lysate preparation

1. If you are starting with a prepared lysate, skip this step but read the note on Page 3 regarding lysis buffers. If you are starting with un-lysed cells or tissue, start here. This is one method by which a lysate for multiomics preps can be prepared, but if it will not work for your sample, contact us and we will help determine an alternative method.
    a. **Before beginning, supplement lysis buffer (LB) with 2-Mercaptoethanol to a final concentration of 284 mM.** For example, add 20 µL of 2-Mercaptoethanol to 980 µL of LB. Only prepare as much LB as you will need the day of use.
    b. For cultured cells:
        i. Add LB supplemented with 2-Mercaptoethanol to your pelleted cells and mix. We recommend adding about 2X the volume of your pellet (ex: if your pellet is 100 µL, add 200 µL LB). More LB may be added to suit your needs.
        ii. If necessary, homogenize the lysate by passing it through a 21-gauge needle up to 10 times.
    c. For tissue samples:
        i. Weigh the tissue sample prior to homogenization. We recommend starting with 5-25 mg of tissue.
        ii. Add LB supplemented with 2-Mercaptoethanol at a ratio of 25 µL LB per 1 mg tissue.
            1. When working with tissue samples, be sure to work as quickly as possible and keep the tissue on ice.
        iii. Homogenize the tissue with a RNase-free pestle for 2-3 minutes for soft tissues and up to 6 minutes for hard tissues. Ensure thorough homogenization.
    d. Assay protein concentration via your method of choice. We recommend a Bradford assay. Note that 2-Mercaptoethanol is incompatible with BCA assays. Protein concentration can be assayed after the ProMTag Multi workflow if working with tissues that require rapid turnaround to avoid RNA degradation. Follow the instructions in Step 2 below.

Protein labeling with ProMTag and binding to ProMTag capture resin

2. **For cultured cells**: Add up to 100 µg of protein in lysis buffer to a low protein binding tube provided with the kit. If the volume of your sample is less than 50 µL, bring the final volume up to 50 µL using the provided lysis buffer.
   For tissue samples: Start with 40 µL of tissue lysate. Add 10 µL LB to bring to a final volume of 50 µL.
   a. If the sample is dilute, a larger starting volume can be used. We recommend keeping the input volume at or under 100 µL. RNA and DNA recovery may be negatively affected by high input volume.
   b. We recommend starting with 100 µg of protein whenever possible. This will maximize DNA, RNA, and peptide yield.
3. Add 4.2 µL ProMTag and pipette up and down gently to mix. Spin briefly to collect the sample at the bottom of the tube.
4. Incubate for 30 minutes at 4°C to produce the ProMTagged sample.
5. During the last 5-10 minutes of the incubation, prepare the ProMTag capture resin for use.
   a. Vortex the ProMTag capture resin to thoroughly resuspend.
   b. Place a RC-tube into a 2 mL waste collection tube and pipette 100 µL of ProMTag capture resin into the RC-tube.
   c. Add 200 µL of ultrapure, nuclease-free water.
   d. Spin briefly (~2 seconds) in a benchtop centrifuge until all the liquid has passed into the waste collection tube. The resin will be bright white when it is dry. Discard the flowthrough and tap the waste tube on a paper towel to empty the waste tube and avoid carryover.
6. Once Steps 4 and 5 are complete, add the ProMTagged sample from Step 4 to the capture resin. Be sure to pipette your sample directly onto the capture resin. Tap the tube gently to mix.
7. Add 175 µL of NAP to the capture resin. Tap gently to mix and be careful to avoid liquid spill through the holes in the RC-tube. Some resin may remain clumped at the bottom of the RC-tube, this is normal.
   a. If your starting volume was higher than 50 µL, adjust the volume of NAP added. Add 175 µL or 3 volumes of NAP, whichever is greater, but do not add more than 300 µL NAP to avoid leakage and sample loss. Nucleic acid recovery will be most efficient when 3 volumes of NAP are added but slightly less NAP can be used.
8. Incubate the ProMTag RC-tube at 4°C with gentle rotation for 30 minutes.
   a. We recommend using a rotisserie to keep the resin suspended, but other methods can be used so long as they are gentle (no harsh shaking).
   b. As the reaction proceeds, the pink solution should turn colorless.

ProMTag capture resin washing and nucleic acid elution

9. Place the RC-tube back into the waste collection tube. Spin briefly (~2 seconds) in a benchtop centrifuge until all of the liquid has passed into the waste collection tube. Discard the flowthrough and tap the waste tube on a paper towel to avoid carryover.
10. Add 200 µL WBN to the sample. Vortex for ~1 second to mix. Spin briefly (~2 seconds) in a benchtop centrifuge until all of the liquid has passed into the waste collection tube. Discard the flowthrough and tap the waste tube on a paper towel to avoid carryover.
11. Repeat Step 10 two more times.
12. If your sample is RNase-rich, we recommend supplementing NAE1 with RNase inhibitor at 2U RNase inhibitor per µL NAE1. Add 60 µL NAE1 to the capture resin. Be sure to pipette NAE1 directly onto the capture resin. Tap the tube gently to mix. Incubate at 4°C with gentle rotation for 5 minutes.
13. Place the RC-tube into a 1.5 mL nucleic acid collection tube. Spin briefly (~2 seconds) in a benchtop centrifuge until all of the liquid has passed into the collection tube. **DO NOT DISCARD THIS.** This is the RNA-enriched eluate, Eluate 1. **Store Eluate 1 on ice.**
14. Add 60 µL NAE2 to the capture resin. Be sure to pipette NAE2 directly onto the capture resin. Tap the tube gently to mix. Incubate at 4°C with gentle rotation for 5 minutes.
15. Place the RC-tube into a new 1.5 mL nucleic acid collection tube. Spin briefly (~2 seconds) in a benchtop centrifuge until all of the liquid has passed into the collection tube. **DO NOT DISCARD THIS.** This is the DNA-enriched eluate, Eluate 2. **Store Eluate 2 on ice.**

Optional. Repeat NAE2 elution (Steps 14 and 15) once and combine Eluates 2 and 3 to increase DNA yield.

Protein release from ProMTag capture resin

16. Place the RC-tube back into the waste collection tube. Add 45 µL WB1. Resuspend one tube of IAA in 20 µL water. Add 5 µL IAA to the capture resin and tap to mix. Incubate at room temperature in the dark for 15 minutes.
17. Add 200 µL WB1 to the capture resin. Vortex for ~1 second to mix. Spin briefly (~2 seconds) in a benchtop centrifuge until all of the liquid has passed into the waste collection tube. Discard the flowthrough and tap the waste tube on a paper towel to avoid carryover.
18. Add 200 µL WB2 to the capture resin. Vortex for ~1 second to mix. Spin briefly (~2 seconds) in a benchtop centrifuge until all of the liquid has passed into the waste collection tube. Discard the flowthrough and tap the waste tube on a paper towel to avoid carryover.
    a. Resin may stick along the sides of the RC-tube after this wash. This is normal.

19. Add 200 µL ultrapure water to the sample. Vortex for ~1 second to mix. Spin briefly (~2 seconds) in a benchtop centrifuge until all the liquid has passed into the waste collection tube. Discard the flowthrough and tap the waste tube on a paper towel to avoid carryover.

20. Repeat Step 19 one more time.

21. Add 50 µL EB to the capture resin. If the ProMTag capture resin is tightly clumped, use a 10 µL pipet tip to gently break apart the clump (do not pipet the resin, simply use the tip to disrupt the clump) and ensure the resin is suspended in the EB.

a. From this stage until elution **do not** centrifuge the sample to bring the liquid back to the bottom of the tube, as doing so will result in loss of proteins or peptides.

22. Incubate the RC-tube at room temperature with gentle rotation for 15 minutes.

Further processing of nucleic acid eluates

23. During Step 22 incubation and/or during the subsequent digestion step, continue processing Eluate 1 and Eluate 2. Depending on your desired downstream application, treat the nucleic acid eluates as necessary. For example, if you are planning on using the nucleic acids for RNA sequencing and whole genome sequencing, we recommend the following steps:

a. Check DNA and RNA concentration in both eluates. We recommend a Qubit dsDNA BR assay for DNA and Qubit RNA BR assay for RNA.

b. If the concentration of DNA in eluate 2 is insufficient for your application, transfer a fraction of eluate 1 into eluate 2.

c. Treat Eluate 1 with DNase (not supplied). This is our preferred protocol for DNase treatment with Ambion™ DNase I (RNase-free):

   i. Add 10X DNase I Buffer to 1X concentration

   ii. Add 4U (2 µL) of DNase I

   iii. Incubate at room temperature for 5 minutes to 1 hour, depending on DNA yield. For ~50 ng DNA, incubate for 5 minutes. For ~250 ng DNA, incubate for 30 minutes. High DNA yield samples (≥ 2 µg) may require more DNase I.

   iv. If necessary, heat inactivate DNase I by adding EDTA to a final concentration of 5 mM. Heat at 75°C for 10 minutes.

d. Treat Eluate 2 with RNase (not supplied). This is our preferred protocol for RNase treatment with Promega RNase A Solution (4 mg/mL).

   i. Add 2 µL of Promega RNase A Solution to Eluate 2.

   ii. Incubate at 37°C for 30 minutes.

e. Store samples at -80°C.

Protein digestion with MT-Trypsin

24. After Step 22 is complete, transfer the RC-tube to a provided low protein binding tube and add 25 µL MT-Trypsin to the ProMTag capture resin. Tap the tube to ensure the resin and sample are mixed and incubate at 37°C for 1 hour.
   a. Longer digestion times will not improve yield. We recommend ensuring that digestion does not exceed 1 hour.

25. Spin briefly (~2 seconds) in a benchtop centrifuge until all the liquid has passed into the low protein binding tube. **DO NOT DISCARD THE FLOWTHROUGH.** This is the Peptide Eluate.

26. Add 50 µL EB to the capture resin and tap to mix.

27. Incubate the RC-tube at room temperature with gentle rotation for 5 minutes.

28. Return the RC-tube to the same Peptide Eluate collection tube from Step 25. Spin briefly (~5 seconds) until all of the liquid has passed into the low protein binding tube.

29. That tube now contains your sample of pure peptides in an volatile, acidic buffer ready for mass spectrometry. If desired, you can concentrate the sample by drying it in a vacuum concentrator.

Quick start guide for ProMTag Multi

For extraction and cleanup of genomic DNA, total RNA, and protein and digestion of extracted protein.
Protocol optimized for up to 100 µg protein with concentrations 1 mg/mL or greater.
This abbreviated guide is intended for users familiar with the ProMTag Multi protocol.
We highly recommend first time users follow the full-length guide.

Note: Never vortex the Resin Capture (RC)-tube alone. Always vortex by placing the RC-tube in a 1.5 mL or 2 mL tube as an adapter to avoid touching the bottom of the tube.

1. Before beginning, supplement the provided lysis buffer (LB) with 2-Mercaptoethanol to a final concentration of 284 mM (20 µL 2-Mercaptoethanol/980 µL LB). Only prepare as much LB as you will need the day of use.
2. Add up to 100 µg of protein sample in up to 50 µL lysis buffer (LB) to a provided 1.5 mL tube.
    a. Consult the full-length guide for sample preparation details.
3. Bring sample volume to 50 µL with LB.
4. Add 4.2 µL ProMTag and pipette to mix. Incubate for 30 minutes at 4°C to produce the ProMTagged sample.
5. During the last 5-10 minutes of incubation, prepare the ProMTag capture resin as follows:
    a. Vortex ProMTag capture resin to resuspend.
    b. Place a RC-tube in a 2 mL waste collection tube and pipette 100 µL of ProMTag capture resin into the RC-tube.
    c. Add 200 µL ultrapure, nuclease-free water and spin briefly to dry resin.
6. Add ProMTagged sample from Step 4 directly to capture resin and tap gently to mix.
7. Add 175 µL (or 3 volumes, whichever is greater) NAP and tap gently to mix. Incubate for 30 minutes with gentle rotation at 4°C.
8. Wash the resin, discarding the flowthrough after each wash:
    a. 3X 200 µL with WBN
9. Add 60 µL NAE1 and tap gently to mix. Incubate for 5 minutes with gentle rotation at 4°C.
    a. If your sample is RNase-rich, we recommend supplementing NAE1 with RNase inhibitor at 2U RNase inhibitor per µL NAE1.
10. Transfer RC-tube to a 1.5 mL tube. Spin briefly to collect the RNA-enriched eluate, Eluate 1. **DO NOT DISCARD**. Store Eluate 1 on ice.
11. Add 60 µL NAE2 and tap gently to mix. Incubate for 5 minutes with gentle rotation at 4°C.
12. Transfer RC-tube to a new 1.5 mL tube. Spin briefly to collect the DNA-enriched eluate, Eluate 2. **DO NOT DISCARD**. Store Eluate 2 on ice.

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a. A second elution with NAE2 can be done to maximize DNA yield if desired.

13. Store the nucleic acid eluates on ice briefly while you continue with the peptide sample preparation. Process nucleic acid eluates as desired for downstream applications. The first eluate will be RNA-enriched, while the second eluate will be mostly DNA. Both eluates will contain DNA. Refer to Step 23 in the full-length manual for more details.

14. Transfer RC-tube to the waste collection tube. Add 45 µL WB1 and 5 µL IAA (resuspend one tube of IAA in 20 µL water). Incubate at room temperature in the dark for 15 minutes.

15. Wash the resin, discarding the flowthrough after each wash:

a. 1X 200 µL with WB1
b. 1X 200 µL with WB2
c. 2X 200 µL with ultrapure water

16. Add 50 µL EB directly to capture resin. If the resin is tightly clumped, use a 10 µL pipette tip to gently break apart the clump and ensure the resin is suspended in EB. Incubate with gentle rotation at room temperature for 15 minutes.

17. Transfer RC-tube to a 1.5 mL low protein binding tube. Add 25 µL MT-Trypsin to the capture resin and tap gently to mix. Incubate at 37°C for 1 hour.

18. Spin briefly to collect the Peptide Eluate. **DO NOT DISCARD**.

19. Add 50 µL EB to the capture resin and incubate with rotation for 5 minutes.

20. Transfer the RC-tube back to the same Peptide Eluate collection tube from Step 18 and spin briefly.

21. Concentrate sample with a vacuum concentrator if desired.