Quick-Start Protocol July 2017

NoviPure® Microbial Protein Kit (50)

All reagents and kit components should be stored at room temperature (15–25°C).

Further information

- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Prepare a working stock of Solution PL prior to each use by adding 4 µl of β-mercaptoethanol (β-ME) per 400 µl of Solution PL. Alternatively, dithiothreitol (DTT) may be added to Solution PL to a final concentration of 1–10 mM. Use a fume hood when using β-ME or DTT.
- Add EDTA-free protease inhibitors to Solution PL. We recommend using Halt™ Protease Inhibitor Cocktail (Thermo Fisher Scientific cat. no. 78429). Use 4 µl of Halt Protease Inhibitor Cocktail per 400 µl of Solution PL. Follow manufacturer's recommendations when using other protease inhibitors.
- Solution PE (elution buffer) contains 1% SDS in HEPES. Some downstream applications may require the removal of SDS. Refer to the handbook for additional information.
- This protocol will co-isolate partially degraded DNA and RNA. Refer to the handbook for additional information about removing nucleic acids.
- Add 0.4 µl of antifoam (provided) to each sample (400 µl) of Solution PL to a final concentration of 0.1%. Vortex antifoam prior to use. Certain microbial cultures such as *E. coli* produce an excessive amount of foam during bead beating. Foam production can cause inconsistent lysis and impacts final protein yields. We recommend adding antifoam regardless of microbial species.

1. Add up to 1.8 ml of microbial (bacteria or yeast) culture to a 2 ml Collection Tube (provided) and centrifuge at 15,000 x *g* for 3 min at room temperature. Completely remove the media supernatant with a pipette tip.
   Note: We recommend using no more than 1×10^8 fungal cells or 1×10^9 bacterial cells per sample.
2. Resuspend the cell pellet in 400 µl of Solution PL (working stock) by vortexing or pipetting. Transfer resuspended cells to the NoviPure Microbial Bead Tube.
3. Secure the NoviPure Microbial Bead Tube horizontally on a Vortex Adapter (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.

4. Centrifuge the tubes at 15,000 x *g* for 1 min at room temperature.
5. Transfer the lysate to a clean 2 ml Collection Tube (provided).
   Note: Expect 200–300 µl. It is normal to transfer some glass beads with the lysate.
6. Add 450 µl of Solution VN to the lysate. Vortex briefly on high to mix.
   Note: The sample may become opaque upon the addition of Solution VN. This is normal
7. Load up to 700 µl of the lysate/Solution VN mix onto an MB Spin Column. Centrifuge at 15,000 x *g* for 1 min at room temperature. **Do not** discard the flow-through.
8. Transfer the MB Spin Column to a clean 2 ml Collection Tube (provided).
9. Add 600 µl of Solution AB to the flow-through from step 7. Vortex briefly on high to mix.
10. Load 650 µl of the mix from Step 9 onto the same MB Spin Column. Centrifuge at 15,000 x *g* for 1 min at room temperature. Discard the flow-through and load the remaining sample volume onto the MB Spin Column. Centrifuge at 15,000 x *g* for 1 min at room temperature and discard the flow-through.
11. Add 650 µl of RNase-free water to the MB Spin Column. Centrifuge at 15,000 x *g* for 1 min at room temperature. Discard the flow-through.
12. Centrifuge the empty MB Spin Column at 15,000 x *g* for 2 min at room temperature.
13. Being careful not to splash any liquid on the filter basket, place the MB Spin Column in a new 2 ml Collection Tube (provided).
    Note: If desired, on-column removal of nucleic acids may be carried out after this step using the protocol provided in the handbook.
14. Add 100 µl of PE Solution to the center of the spin filter membrane. Incubate for a minimum of 1 min at room temperature.
    Note: Depending on the downstream application, alternate elution buffers, such as urea:thiourea or cleavable detergents, may be used. Using alternative elution buffers may result in reduced protein recovery. Refer to the handbook for additional information.
15. Centrifuge at 15,000 x *g* for 1 min at room temperature.
16. Discard the MB Spin Column. The sample is now ready for downstream applications.
    Note: For 2D SDS-PAGE and in-solution proteolytic digestion for mass spectrometry, removal of 1% SDS may be required. Refer to the handbook for additional information.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. Trademarks: QIAGEN®, Sample to Insight®, NoviPure® (QIAGEN Group); Halt™ (Thermo Fisher Scientific). 1104523 07/2017 HB-2245-001 © 2017 QIAGEN, all rights reserved.