Quick-Start Protocol May 2017

# AllPrep® Fungal DNA/RNA/Protein Kit

All reagents and kit components of the AllPrep Fungal DNA/RNA/Protein Kit 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

- Add 100% ethanol (user provided) to Solutions IW and Solution WP as indicated on the bottle label.
- Add 100% isopropanol (user provided) to Solution RW as indicated on the bottle label.
- To prepare Solution HC working stock, add 3.5 µl of β-mercaptoethanol (β-ME) per 350 µl of Solution HC. Alternatively, dithiothreitol (DTT) may be added to Solution HC to a final concentration of 1–10 mM. Use a fume hood when using β-ME or DTT.
- We strongly recommend using Halt™ Protease Inhibitor Cocktail (Thermo Fisher Scientific cat. no. 78429). Use 3.5 µl of Halt Protease Inhibitor Cocktail per sample. Follow the manufacturer's recommendations when using other protease inhibitors.

1. Add 1.8 ml of fungal culture to a 2 ml Collection Tube (provided). Centrifuge for 3 min at 15,000 x *g*. Remove all of the supernatant with a pipette tip.
   **Note:** We recommend using no more than $1x10^8$ fungal cells per sample.
2. Re-suspend the cell pellet in 350 µl of Solution HC (working stock) by vortexing or pipetting. Transfer the re-suspended cells to a PowerBead Tube.
   **Note:** Solution HC must be freshly prepared with β-ME (or DTT) and protease inhibitors.
3. Secure the PowerBead Tube horizontally using a Vortex Adapter (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.
4. Quick-spin the PowerBead Tube. Remove the cap and add 175 µl of Solution MR directly to the PowerBead Tube. Recap and vortex on high for at least 10 s to mix.
5. Centrifuge the PowerBead Tube at 15,000 x *g* for 2 min at room temperature.

**DNA Purification**

6. Transfer 350 µl of lysate from the Tube directly to an MB Spin Column. Centrifuge for 1 min at 15,000 x *g*. Save the flow-through for RNA purification (Step 14).
   **Note:** It is normal to transfer some glass beads with the lysate.
7. Transfer the MB Spin Column to a clean 2 ml Collection Tube (provided).

8. Add 650 µl of Solution EA and centrifuge at 15,000 x *g* for 1 min. Discard flow-through.
9. Add 650 µl of Solution IW and centrifuge at 15,000 x *g* for 1 min. Discard flow-through.
10. Centrifuge at 15,000 x *g* for 2 min. Being careful not to splash liquid on the filter basket, place the MB Spin Column in a new 2 ml Collection Tube (provided).
11. Add 100 µl of Solution EB to the center of the white filter membrane.
12. Incubate for 1 min at room temperature. Centrifuge at 15,000 x *g* for 1 min.
13. Discard the MB Spin Column. The DNA is now ready for downstream applications.

**RNA purification**

14. Add 350 µl of Solution RB to the flow-through from Step 6. Vortex briefly on high.
15. Add the lysate to a new MB Spin Column and centrifuge at 15,000 x *g* for 1 min. Save the flow-through for protein purification (Step 23).
16. Transfer the MB Spin Column to a clean 2 ml Collection Tube (provided).
17. Add 650 µl of Solution RW. Centrifuge at 15,000 x *g* for 1 min. Discard flow-through.
18. Add 650 µl of 100% ethanol (user provided). Centrifuge at 15,000 x *g* for 1 min. Discard the flow-through.
19. Centrifuge at 15,000 x *g* for 2 min. Being careful not to splash liquid on the filter basket, place the MB Spin Column in a new 2 ml Collection Tube (provided).
20. Add 100 µl of RNase-free water (provided) to the center of the white filter membrane.
21. Incubate for 1 min at room temperature. Centrifuge at 15,000 x *g* for 1 min.
22. Discard the MB Spin Column. The RNA is now ready for downstream applications.

**Protein purification**

23. Add 650 µl of Solution AB to the flow-through from Step 15. Vortex briefly on high.
24. Load up to 650 µl onto a new MB Spin Column and centrifuge at 15,000 x *g* for 1 min.
25. 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  and discard the flow-through.
26. Add 650 µl of Solution WP. Centrifuge at 15,000 x *g* for 1 min. Discard flow-through.
27. Centrifuge at 15,000 x *g* for 2 min. Being careful not to splash liquid on the filter basket, place the MB Spin Column in a new 2 ml Collection Tube (provided).
28. Add 100 µl of Solution PE to the center of the white filter membrane.
29. Incubate for 1 min at room temperature. Centrifuge at 15,000 x *g* for 1 minute.
30. 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 the 1% SDS may be required. Refer to the Handbook for more information.