May 21, 2024

MP Biomedicals FastDNA™ SPIN Kit

This protocol is a draft, published without a DOI.

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Protocol Citation: Karen Keegan 2024. MP Biomedicals FastDNA™ SPIN Kit. **protocols.io https://protocols.io/view/mp-biomedicals-fastdna-spin-kit-dd3j28kn**

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

Created: May 21, 2024

Last Modified: May 21, 2024

Protocol Integer ID: 100171

Abstract

MP Biomedicals FastDNA™
SPIN Kit for feces

Materials

FASTDNA Spinkit for feces

MP Biomedicals FastDNA™ SPIN Kit for feces

1 In a 2 mL Lysing Matrix E tube, add 500 mg feces sample, 825 µL Sodium Phosphate Buffer, and 275 µL of PLS solution. Shake to mix. Vortex 10-15 seconds.

2 Centrifuge samples at 14,000 x g for 5 minutes and decant supernatant.

3 Add 978 µL Sodium Phosphate Buffer and 122 µL MT Buffer.
Shake vigorously or vortex briefly to mix.

4 Homogenize samples in the FastPrep 24 instrument at setting 6.0m/s for 40 seconds.

5 Centrifuge samples at 14,000 x g for **15 minutes.** NOTE: Extending centrifugation to 15 minutes (did 15 instead of 5) can enhance elimination of excessive debris from large samples, or from cells with complex walls.

6 Transfer the supernatant to a clean 2.0 mL centrifuge tube.

7 Add 250 µL of PPS solution, shake vigorously to mix, and incubate at 4°C for 10 minutes. Do not vortex! Centrifuge samples at 14,000 x g for 2 minutes.

8 While samples are centrifuging, add 1 mL of Binding Matrix Solution to a clean 15 mL conical tube (not supplied).

9 Transfer supernatant to the Binding Matrix Solution in the 15 mL conical. Shake gently by hand to mix, then place on a shaker/rocker for 3-5 minutes.

10 Centrifuge samples at 14,000 x g for 2 minutes. Decant the supernatant

11 Wash the binding mixture pellet by gently resuspending with 1 mL Wash Buffer #1.

12 The following step will require two spins. First, transfer approximately 600 µL of the binding mixture to a SPIN Filter tube and centrifuge at 14,000 x g for 1 minute. Empty the catch tube. Add the remaining

binding mixture to the SPIN Filter tube and centrifuge as before. Empty the catch tube again.

13 Add 500 µL of prepared Wash Buffer #2 to the SPIN Filter tube and gently resuspend using the force of the liquid from the pipette tip to resuspend the pellet. Do not vortex. NOTE: Ensure that ethanol has been added to the Wash Buffer #2

14 Centrifuge samples at 14,000 x g for 2 minute. Discard the flow-through.

15 Centrifuge the sample again for 2 minutes to extract residual ethanol from the binding matrix and dry the sample.

16 Transfer the SPIN Filter bucket to a clean 1.9 mL Catch Tube. Add 60-100 µL TES. Flick the tube or stir the matrix with a pipette tip to resuspend the pellet. Do not vortex.

17 Centrifuge samples at 14,000 x g for 2 minutes to elute purified DNA into the clean catch tube. Discard the SPIN filter. Store at -80°C until use.