SPINeasy 96-Well DNA Pro Kit for Soil

Cat. No.: 116546096 (96 PREPS) /116546496 (4 x 96 PREPS)

Quick-Start Protocol

Revision 1.0 Mar 2024

Scan QR code for more information from instruction manual

Notes before starting:

- Add 70 mL of isopropanol (for 96 preps kit) or 266 mL of isopropanol (for 4 x 96 kit) into **Buffer S3** and mark the bottle.
- Add 100 mL of absolute ethanol (for 96 preps kit) or 360 mL of absolute ethanol (for 4 x 96 kit) into **Buffer S5** and mark the bottle.
- If Buffer S1 has precipitate, heat at 37° C until precipitate dissolves.
- Upon receiving the kit, Buffer S2 needs to be stored at 2-8° C.
- Step 5 requires the use of a swing bucket centrifuge capable of generating at least 4,000 *g* to obtain optimal results. Use the maximum speed available if 4,000 *g* is not feasible.
- Seal 96 DNA plate G after each buffer loading to avoid cross-contamination.
- This kit can also be used with a vacuum manifold during binding and washing steps (see below).

Homogenize

1. Weigh the soil sample(250-500 mg) and load into the **Lysing Matrix YB** tube, add **900 µL Buffer S1 and 20-25 µL RNase A.**
2. Homogenize with a FastPrep® instrument, at speed setting of **5 m/s, 35 s** or vortex for **20 min @ 2,600-3,000 rpm**. Centrifuge for **2 min @≥ 15,000 *g*.**

Remove inhibitor

3. Transfer the supernatant (~700 µL) into a new 2 mL tube (not provided).
   ***Optional:**. The Lysing Matrix YB tube may be re-centrifuged after transfer of the supernatant to recover any additional lysate using a tip*
   Add **400 µL Buffer S2**, invert and mix 5 times and centrifuge for **2 min @≥ 15,000 *g*.**

Bind

4. Transfer the supernatant (~800 µL) into a new 2 mL tube (not provided). Add **800 µL Buffer S3**, invert and mix 5 times. The subsequent steps can be performed using either centrifuge or vacuum manifold .

Centrifuge

5. Put the **96 DNA Plate G** on a Deep-well Plate (provided) and transfer the lysate mixture (~800 µL) into each well of the 96 DNA Plate G. Seal the plate with a **Sealing Film** (provided) and centrifuge for **2 min @ 4,000 *g*.** Discard the flow-through. Repeat the process until all the lysate has passed through.

Wash

6. Remove the seal and add **800 µL Buffer S4** into each well of the 96 DNA Plate G. Seal the plate and centrifuge for **2 min @ 4,000 *g*.** Discard flow through and place the 96 DNA Plate G back into the same Deep-well Plate.
7. Remove the seal and add **800 µL Buffer S5** into each well of the 96 DNA Plate G. Seal the plate and centrifuge for **2 min @ 4,000 *g*.** Discard flow through and place the 96 DNA Plate G back on the same Deep-well Plate.
8. Dry the 96 DNA Plate G by centrifuging for **10 min @ >4,000 *g*.**

Vacuum manifold

5. Put the **96 DNA Plate G** on the vacuum manifold (with the waste tray). Transfer the lysate mixture(~800 µL) into each well of the 96 DNA Plate G. Apply vacuum until all supernatant passed through into the waste tray (repeat this step once).
6. Add 800 µL Buffer S4 into each well of the 96 DNA Plate G. Apply vacuum until all buffer passed through into the waste tray.
7. Add 800 µL Buffer S5 into each well of the 96 DNA Plate G. Apply vacuum until all buffer passed through into the waste tray.
8. Apply vacuum for another 10 min to remove any residual wash buffer from the wells. Tap the plate on clean paper towel to dry the plate as much as possible.

Elute

9. Transfer the 96 DNA Plate G onto a clean **Elution plate** (provided). Add **100-120 µL Buffer S6** directly onto the membrane of the 96 DNA Plate G. Incubate for **2 min @** room temperature and centrifuge for **5 min** @ **4,000 *g*.** Seal the DNA Elution plate containing the eluted DNA with a Sealing Film.
   ***Optional:** Eluting with 120 µL of Buffer S6 will maximize nucleic acid yield. For a more concentrated sample, a minimum of 100 µL Buffer S6 can be used.*

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- Flow chart

Homogenize

Soil 250-500 mg

Buffer S1 (900 µL) + RNase A (20-25 µL)

5 m/s, 35 s or 20 min 2,600-3,000 rpm

Remove inhibitor

2 min@≥15,000 g

Transfer supernatant (~700 µL) into 2 mL tube

Buffer S2 (400 µL)

mix 5x

Bind

2 min@≥15,000 g

Transfer supernatant (~800 µL) into 2 mL tube

Buffer S3 (800 µL)

mix 5x

Lysate

Centrifuge	Vacuum manifold
Transfer 800 µL mixture	Transfer 800 µL mixture
2 min@4,000 xg	

Wash

Centrifuge	Vacuum manifold
Buffer S4 (800 µL)	Buffer S4 (800 µL)
2 min@4,000 xg	
Buffer S5 (800 µL)	Buffer S5 (800 µL)
2 min@4,000 xg	
Drying *10 min@ > 4,000 xg*	*Vacuum dry for 10 min*

Elute

Buffer S6 (100-120 µL)

Incubate for 2 min
5 min@4,000 xg

Highly purified genomic DNA