

SoilMaster™ DNA Extraction Kit

Cat. Nos. **SM02050**, **SC04350** and **SR04350**

The **SoilMaster™ DNA Extraction Kit** provides all of the reagents necessary to recover PCR-ready DNA from a variety of environmental samples. The kit utilizes a hot detergent lysis process^{1,2} combined with a chromatography step, which removes enzymatic inhibitors known to co-extract with DNA from soil and sediment samples.^{3,4} The extracted DNA is PCR-ready, and can be used with the FailSafe™ PCR System to amplify bacterial, plant or fungal templates.

Product Specifications

Storage: Store the Proteinase K and DNA Precipitation Solution at -20°C in a freezer without a defrost cycle. Store the remainder of the kit components at room temperature.

Storage Buffer: Proteinase K is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 10 mM CaCl₂, 0.1% Triton® X-100 and 1mM dithiothreitol.

Quality Control: The SoilMaster DNA Extraction Kit is function-tested by assaying extracted soil sample DNA by PCR and agarose gel electrophoresis.

Related Products: The following products are also available:

- MasterPure™ Complete DNA and RNA Purification Kit
- MasterPure™ DNA Purification Kit
- MasterPure™ RNA Purification Kit
- MasterPure™ Plant Leaf DNA Purification Kit
- MasterPure™ Yeast DNA Purification Kit
- FailSafe™ PCR System
- MasterAmp™ PCR Optimization Kits
- MasterAmp™ *Taq*, *Tth*, *Tfl* and AmpliTherm™ DNA Polymerases
- BuccalAmp™ DNA Extraction Kit

SoilMaster™ DNA Extraction Kit Contents

The SoilMaster™ DNA Extraction Kit contains sufficient reagents to perform 50 extractions. The kit contains the following reagents: Cat. No. SM02050

Soil DNA Extraction Buffer.....	12.5 ml
Proteinase K (50 µg/µl)	100 µl
Soil Lysis Buffer	2.5 ml
Protein Precipitation Reagent.....	3 ml
Inhibitor Removal Resin.....	55 ml
Spin Columns.....	50
DNA Precipitation Solution	300 µl
Pellet Wash Solution *	15 ml
TE Buffer	15 ml
(10 mM Tris-HCl [pH 7.5], 1 mM EDTA)	

Also available:

Spin Columns	
Cat. No. SC04350.....	50
Inhibitor Removal Resin	
Cat. No. SR04350.....	55 ml

* **Note:** Ethanol must be added to the Pellet Wash Solution before its first use (see Preparation of Pellet Wash Solution on page 3).

If a cap is desired for later spins of the column, the original cap can be cut off its tube and substituted for the cap of other 1.5 ml tubes.

References:

1. Selenska, S. and Klingmuller, W. (1991) *Letters in Appl. Microbiol.* **13**, 21.
2. Zhou, J. *et al.*, (1996) *Appl. Environ. Microbiol.* **62**, 316.
3. Tsai, Y. L. and Olson, B.H. (1992) *Appl. Environ. Microbiol.* **58**, 754.
4. Tebbe, C.C. and Vahjen, W. (1993) *Appl. Environ. Microbiol.* **59**, 2657.

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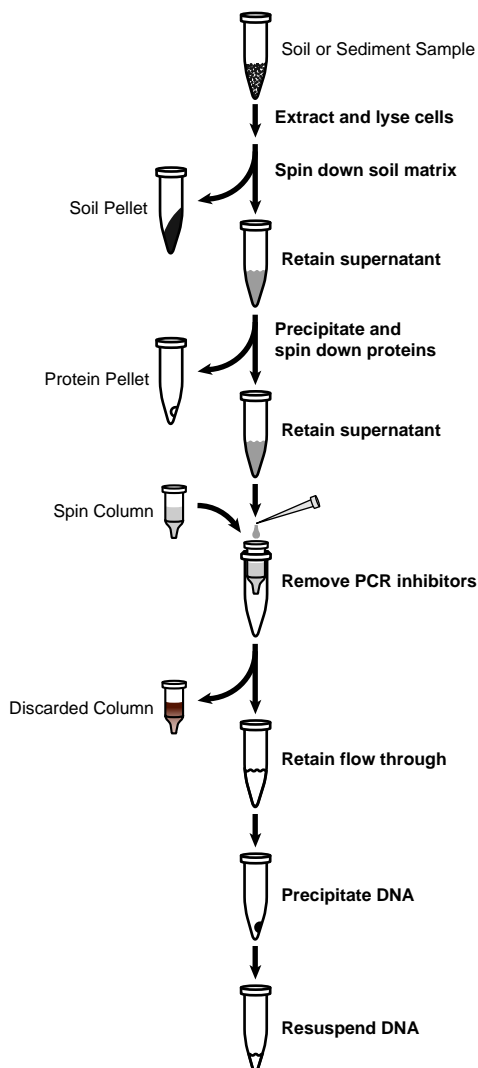
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General Considerations

- Sample Sources:** We have used this kit to isolate PCR-ready DNA from a variety of soil and sediment sources including: forest soil, marsh soil, garden soil and cave sediment. Due to the wide variety of organic contaminants in different soil samples, some optimization of the initial sample size used and the amount of extract loaded onto the spin column may be required.
- Sample Size:** Generally 100 mg of soil will provide 300 μ l of PCR-ready DNA. Users can extract DNA from larger sample sizes by using multiple spin columns. Do not load more than 150 μ l of extract on a column.
- Quantitation of DNA:** If necessary, the concentration of PCR-ready DNA extracted by the SoilMaster DNA Extraction Kit should be measured by comparison to a standard amount of genomic DNA run side-by-side on an agarose gel. The remaining organic compounds co-extracted with the DNA may interfere with fluorimetry and spectrophotometry.

Figure 1. An overview of the process for extraction of DNA from soil samples.



Soil DNA Purification Protocol

A. Preparation:

Spin Columns

1. Add 550 μ l of Inhibitor Removal Resin to each empty Spin Column to be used. Centrifuge for 1 minute at 2000 x g to pack the column.
2. Decant flow-through and place the column in the same collection tube.
3. Add another 550 μ l of Inhibitor Removal Resin to each packed column. Centrifuge for 2 minutes at 2000 x g.
4. Move the column to a clean 1.5 ml collection tube.

Pellet Wash Solution

1. Add 45 ml of ethanol to the Pellet Wash Solution **before first use**.

B. Cell Lysis:

1. Weigh out 100 mg of the soil sample into a 1.5 ml tube.
2. Add 250 μ l of Soil DNA Extraction Buffer and 2 μ l of Proteinase K; vortex briefly.
3. **(Optional)** To increase the yield of DNA, shake the tube at 37°C for 10 min. or vortex for 2 min. Note: vortexing may shear the DNA.
4. Add 50 μ l of Soil Lysis Buffer and vortex briefly.
5. Incubate at 65°C for 10 minutes.
6. Centrifuge for 2 minutes at 1000 x g.
7. Transfer 180 μ l of the supernatant to a new tube.
8. Add 60 μ l of Protein Precipitation Reagent, mix thoroughly by inverting the tube.
9. Incubate on ice for 8 minutes. Centrifuge the tube for 8 minutes at maximum speed.
10. Carefully transfer 100-150 μ l of the supernatant directly onto the prepared Spin Column (from Section A).
11. Centrifuge for 2 minutes at 2000 x g into the 1.5 ml tube. Discard the column.
12. Add 6 μ l of DNA Precipitation Solution, vortex briefly. Incubate the tube at room temperature for 5 minutes.
13. Centrifuge for 5 minutes at maximum speed. Carefully decant the supernatant.
14. Wash the pellet with 500 μ l of Pellet Wash Solution (prepared in Section A). Invert to mix then spin for 3 minutes at maximum speed. Carefully decant the supernatant.
15. Repeat the wash and spin.
16. Resuspend the pellet in 300 μ l of TE Buffer.

Troubleshooting DNA Extractions

DNA does not amplify by PCR

- 1) Optimize cycling conditions.** Decrease the annealing temperature of the cycling profile by 2 degrees or more. Some primer pairs require a lower annealing temperature (less stringent conditions) when amplifying soil DNA.
- 2) Use less starting material.** Some environmental samples contain significantly larger amounts of enzymatic inhibitors. When using these samples, begin the extraction with less starting material (50 mg).
- 3) Load less extract onto the column.** If any color remains in the extract after the Inhibitor Removal Spin Column step, load less extract onto the column.
- 4) Dilute the extracted DNA.** Dilute the extracted DNA 2-10 fold before amplification to decrease the effects of any remaining enzymatic inhibitors.
- 5) Rewash the pellet with the Pellet Wash Solution.** This step is important in removing residual inhibitors of DNA amplification.

DNA is sheared

- 1) Eliminate the vortex mixing step.** Eliminate the 2 minute vortex mixing step when extracting the DNA. Shake at 37°C instead or simply skip this step entirely. The yield of DNA will be decreased, but the integrity of the DNA will be significantly improved and hundreds of PCR amplifications can be performed on the resulting DNA.