

# **Metagenom Bio**

# Designed with the Microbiome in Mind

# **Sox DNA Isolation Kit**

Product catalog No. 18011-50

Quantity: 50 preps

User Manual

Version: 14.7

550 Parkside Dr. Unit A9 Waterloo, ON N2L 5V4 Canada

Phone: +1 844 973 4363 info@metagenom.com

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### Introduction

Sox DNA Isolation Kit is a rapid and efficient method for extraction of microbial DNA from a variety of soil samples. Microbial cells are lysed with chemicals and mechanical methods. Contaminants are removed from the resulting lysate. DNA is then purified using a silica spin column. Organic solvents and proteinases are not used. This protocol has been validated by successful extraction of DNA from a variety of soil samples and high-resolution 16S rRNA gene profiles of DNA preps.

#### Kit contents

	Kit catalog #18011-50	
Component	Catalog #	Amount
Bead tubes	18011-501	50
Solution Sox1	18011-502	60 ml
Solution Sox2	18011-503	5 ml
Solution Sox3	18011-504	2 × 63 ml
Solution Sox4 (concentrate)*	18011-505	14 ml
Solution Sox5	18011-506	10 ml
Spin columns with 2 ml collection tubes	18011-507	50
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<sup>\*</sup>Add 56 ml of 96-100% ethanol before use.



#### Kit storage

Kit reagents and components should be stored at room temperature. Solution Sox3 should be kept away from light. If precipitate occurs in Solution Sox1 and Sox3, warm the solutions at 65°C with occasional mixing until the precipitate is completely dissolved.

#### **Precautions and disclaimers**

This kit is designed for research only, and not for diagnostic use. Please wear a lab coat and gloves to avoid skin contact with reagents in this kit. Do not mix Solution Sox3 or extraction waste with bleach or any other form of acid. Solution Sox4 should be kept away from open flames and sparks after adding ethanol. For more information, please consult the Material Safety Data Sheets available at https://shop.metagenom.com/pages/resources.

## Equipment, reagents and consumables supplied by users

- Microcentrifuge (20,000 × g)
- Vortexer or bead beater
- Pipettors
- o Pipettor tips
- o 1.5 ml DNase-free microcentrifuge tubes
- o 96-100% ethanol



#### **User Protocol**

All procedures are performed at room temperature unless otherwise stated. Solution Sox4 (concentrate) must be diluted with 56 ml of 96-100% ethanol before use.

- 1. Add 0.25 grams of soil sample into a provided bead tube.
- 2. Add 850 μl of **Solution Sox1** to the tube and mix by inverting the tube 5 times. Caution: If white precipitate occurs in **Solution Sox1**, warm it to 65°C for 5 minutes or until the precipitate is completely dissolved before use. The solution can be used when it is still warm. If a sample has high water content, transfer beads from the bead tube to a 1.5 ml microcentrifuge tube (not provided), add wet sample to the empty bead tube, centrifuge at 12,000 × g for 5 minutes, and remove the liquid with a pipette tip. Transfer the glass beads back to the bead tube, and then add **Solution Sox1**.
- 3. Secure the bead tube horizontally on a flat-bed vortex pad with tape or on a vortex microtube holder. Vortex at maximal speed for 10 minutes. Alternatively, beat for 40 seconds at 6M/S using FastPrep®-24 instrument (MP Biomedicals) or other commercial bead beaters according to recommendations from the manufacturer.
- 4. Centrifuge the tube at  $12,000 \times g$  for 5 minutes.
- 5. Transfer up to 550 μl of supernatant to a 1.5 ml microcentrifuge tube (not provided). If removal of RNA is required, add 10 μl of DNase-free RNase A (10 mg/ml, not provided) to the tube, mix by gently pipetting 3 times, and incubate for 10 minutes at room temperature.



- Add 55 μl of Solution Sox2 to the supernatant, mix by inverting the tube 5 times.
- 7. Incubate the tube on ice for 5 minutes.
- 8. Centrifuge the tube at  $20,000 \times g$  for 5 minutes.
- 9. Transfer 500  $\mu$ l of supernatant, avoiding transfer of any of the pellet material, to a clean 1.5 ml microcentrifuge tube (not provided). Caution: If the supernatant is unclear, transfer all solution to a new 1.5 ml microcentrifuge tube, centrifuge at 20,000  $\times$  g for 3 minutes to clarify the solution, and then transfer 500  $\mu$ l of supernatant to a new 1.5 ml microcentrifuge tube.
- 10. Add 1000 μl of **Solution Sox3** to the supernatant and mix by pipetting 3 times.
- 11. Load 550  $\mu$ l solution into a spin column and centrifuge at 10,000  $\times$  g for 1 minute. Discard the flow through and add an additional 550  $\mu$ l of solution to the spin column and centrifuge at 10,000  $\times$  g for 1 minute. Load the remaining solution to the column and centrifuge at 10,000  $\times$  g for 1 minute. Discard the flow through.
- 12. Optional: if the sample has high content of humic acids, add 550 μl of Solution Sox3, wait for 2 minutes and centrifuge at 14,000 × g for 1 minute.
  Discard the flow through. Repeat this step once.
- 13. Add 550  $\mu$ l of **Solution Sox4**, wait for 2 minutes and centrifuge at 14,000 × g for 1 minute. Discard the flow through. Repeat this step once.
- 14. Centrifuge the column in the collection tube at  $14,000 \times g$  for 2 minutes.
- 15. Place the column in a 1.5 ml microcentrifuge tube (not provided).
- 16. Add 100 µl of **Solution Sox5** to the center of white membrane.



- a. Optional: Add 35-50 μl of Solution Sox5 instead if a higher concentration of DNA is desired.
- 17. Wait for 2 minutes and then centrifuge at  $10,000 \times g$  for 1 minute.
- 18. Discard the spin column. The DNA is now ready for downstream applications.

# **Assessing DNA yield**

The amount of DNA can be estimated by Nanodrop spectrophotometer using Solution Sox5 as a blank. Agarose gel electrophoresis can be employed to verify the DNA content and evaluate DNA shearing and/or degradation. In addition, fluorometric quantification of DNA can be used if metagenomic DNA sequencing is desired.

### **DNA** storage

Solution Sox5 does not contain EDTA. We recommend storing DNA prep at -20° to -80°C.

### **Troubleshooting guide**

Problem	Cause	Solution
Low	Presence of humic acids	Wash the column with extra Solution
$A_{260}/A_{280}$ or		Sox3, wait for 2 minutes and then
$A_{260}/A_{230}$		centrifuge 2 minutes
ratio		
	Salt contamination	Ensure the column is dried before elution
	Low DNA concentration	Concentrate DNA prep
	Low amount of DNA in soil	Use a few more bead tubes for one
	sample	sample
		Concentrate DNA prep with ethanol
		precipitation or other methods
Low DNA	Incomplete homogenization	Ensure to vortex the sample thoroughly.
Yield		
	Cells difficult to lyse	Incubate the bead tube at 70°C for 10 min
		after adding Solution Sox1, followed by
		vortexing or beating
		volutioning of beauting



	DNA washed off  Inhibitory substance in the eluted DNA	Solution Sox4 must be diluted with 96-100% ethanol before use  Wash the column with extra Solution Sox3, wait for 2 minutes and then centrifuge 2 minutes
Poor downstream applications	Excess amount of DNA inhibits PCR	Check the amount of DNA by agarose gel electrophoresis or fluorometric quantification
	Residual ethanol in DNA prep	Dilute DNA prep (1:10-100)  Completely dry the column before elution
Little	Sample too dry	Add more Solution Sox1 to bead tube, or
	Sample too dry	
supernatant		to the supernatant
after first	I	Charle the containing of the c
centrifuge	Insufficient centrifugal	Check the centrifugal force and
step	force	increase the time of centrifugation
DNA shearing	DNA degraded	Ensure soil sample is stored properly, and DNase-free working environment
	Vortexing too long	Shorten the vortexing period to less than 5 minutes
Silvaring		Incubate the bead tube at 70°C for 10
		minutes, vortex for 30 seconds, heat
		another 10 minutes, vortex for 30
		seconds. Please note that this procedure
		decreases DNA shearing, but also
		reduces yield.

Thank you for using our Sox DNA Isolation Kit. Please contact our technical support (info@metagenom.com) if you have any questions, we are at your service.

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Phone: +1 844 973 4363 www.metagenom.com

