コスモ・バイオ株式会社

BIOO SCIENTIFIC™

MAXIMIZE SCIENCE FOR LIFE

BIOO FOOD AND FEED SAFETY

MAX SIGNAL™

Silica Filter Bacterial DNA Extraction Kit Manual

Catalog # 3805-01

Reference #: -----

TABLE OF CONTENTS

GENERAL INFORMATION

Product Description

The *MaxSignal™ Silica Filter Bacterial DNA Extraction Kit* contains reagents and materials for purification of chromosomal DNA from liquid cultures or isolated colonies of gram positive and gram negative bacteria. The purification method is based on lysis of pelleted bacteria at high temperature followed by solid-phase extraction of the DNA on silica filters. The procedure can be carried out in less than 30 min using common laboratory equipment. The purified DNA is especially useful as template for PCR. Note, the kit is not designed for extraction of plasmid DNA from bacteria.

Summary of Kit Features

- Purification procedure is rapid since no protease digestion is required
- No organic solvents such as phenol or chloroform are required
- No alcohol precipitation required
- Kit components are stable at ambient temperature
- Flexible elution protocol can be adjusted according to user requirements

Kit Contents, Storage and Shelf Life

The *MaxSignal™ Silica Filter Bacterial DNA Extraction Kit* contains reagents for 50 extractions of DNA from liquid cultures or from colonies growing on agar plates. The shelf life of the kit is at least 12 months when the components are properly stored.

Kit Contents	Amount	Storage
Lysis Solution	28 mL	Room temp
Binding Buffer	18 mL	Room temp
Wash Solution Concentrate (note, add 58 mL ethanol to make working Wash Solution)	24 mL	Room temp
Silica Filter Basket/Collection Tube Assemblies	50 each	Room temp
Collection Tubes for DNA Elution	50 each	Room temp
DNA Elution Solution	10 mL	Room temp

Required Materials Not Provided With the Kit

- Microcentrifuge
- Heat Block and freezer or container of ice
- Ethanol, 99% - 100%, ACS grade or equivalent
- Vortex mixer
- 1.5 ml microfuge tubes (nuclease-free)
- High-volume and medium-volume pitettors and tips; note, barrier tips are recommended
- Disposable gloves to prevent contact with kit components

Safety Precautions

- Use standard precautions to avoid contact with potentially infectious material
- Wear gloves to avoid skin contact with kit reagents

BACTERIAL DNA PURIFICATION PROTOCOL

A. Prepare liquid culture of bacteria in appropriate media. Typically cultures are grown to stationary phase by incubating overnight at 37°C with shaking, in a flask at least 4 times the volume of the liquid culture. Conditions may be adjusted depending on the type of bacteria and whether DNA is desired from bacteria in the stationary vs logarithmic phase of growth.

B. Alternatively, extraction may be carried out using colonies grown on agar plates

Before beginning procedure:

- Make working Wash Solution by adding 58 mL of ethanol (99% - 100%) to the Wash Solution Concentrate. Mix well and store at room temp.
- Prepare a heat block at 95°C

1. Spin the 1 – 1.5 mL of enriched culture for ~ 30 sec at ~ 10,000 rpm to pellet bacteria. Thoroughly remove supernatant. Pellets may be stored at -20°C. If using bacteria from agar plate, scrape one or more colonies from the plate using a suitable instrument, for example a sterile inoculating loop.
2. Add 0.5 mL Lysis Solution to the pellet and resuspend pellet by vortexing. If sample was scraped from an agar plate, swirl the scraped bacteria in a tube containing 0.5 ml Lysis Solution.
3. Incubate prep for 5 min in 95°C heat block. Be sure the tube is tightly capped. (Note, during this step, heat ~ 100 μL of Elution Solution per prep in a tightly closed microfuge tube.)
4. Add 0.3 mL of Binding Buffer and mix well. A flocculent white precipitate should form.
5. Store the prep at -20°C (or on ice) for 5 min.
6. Spin the prep for 3 min at 12,000 rpm (~12,000 x g). The precipitate should form a large tight pellet.
7. Remove clear supernatant to a new tube, noting the volume. Expected volume is ~ 0.65 mL.
8. Add 0.4 volumes of ethanol, 99% - 100% (expected volume of ethanol to add is ~0.26 mL) and mix well by vortexing for ~ 5 seconds. Prep should be clear.
9. Pass the prep over a silica filter by loading ~ 0.7 mL of prep into the Silica Filter Basket / Collection Tube assembly and spinning for ~20 sec at ~12,000 rpm. Discard filtrate and replace filter basket into collection tube. Repeat this process to filter remaining prep (~200 μL).

   Note, the exact time and rpm used for centrifugation in this and subsequent steps is not critical. Use time and rpm as needed to pass all the liquid through the silica filter. In general, longer centrifugation times and/or higher rpm are needed to filter preps from cultures grown to higher saturation levels.
10. Wash filter with 0.7 mL of Wash Solution. Add the Wash Solution to the filter basket / collection tube assembly and spin ~ 20 sec , then discard filtrate and replace filter basket into collection tube. (Note, be sure that the Wash Solution concentrate was mixed with ethanol as described above.)
11. Repeat wash step with additional 0.7 mL Wash Solution. After discarding filtrate, replace cartridge and spin for 20 sec at 12,000 rpm to dry the filter.
12. Transfer cartridge to clean 2 mL collection tube and add ~50 - 100 μL of DNA Elution Solution which has been preheated to ~75 - 95 °C to the center of the filter. Close the lid and store assembly at room temp for ~ 1 min. Note, the exact volume of elution solution is not critical and may be adjusted to user preference. Use more elution solution for preps from bacteria that grow to high density in saturated cultures. Use less elution solution if more highly concentrated DNA is desired.

13. Spin tube for ~ 20 sec at 12,000 rpm to elute the nucleic acid.

14. Optional: to more completely recover the DNA from preps using high levels of bacteria, repeat the elution step. Add a second aliquot of DNA Elution Solution to the filter and centrifuge as before, recovering the eluted DNA in the same tube. Alternatively, to maximize DNA recovery without diluting the prep, remove the filter basket and aspirate the eluted nucleic acid into a pipet tip, then replace the filter and dispense the eluate onto the center of the filter. Centrifuge as before. Discard filter.

Store the DNA at -20°C. If desired, analyze ~ 20 μL of the prep on a native agarose gel. A band of high molecular weight ethidium-staining material is usually visible. Occasionally, additional smaller bands (bacterial ribosomal RNA) may be visible. The DNA may not be detectable by agarose gel analysis if the density of bacterial growth in the culture was low, however in that case the presence of bacterial DNA can usually be verified using sensitive PCR-based methods.

Troubleshooting

PROBLEM	SOLUTION
Filter clogs when passing the prep over the filter at Step 9	Increase time and/or speed of centrifugation. In subsequent preps, use less culture and/ or reduce incubation time to minimize culture density.
Interference from RNA in the eluted DNA	If RNA contamination interferes with use of the DNA, treat the eluted DNA with RNase A. For example, add 1 – 5 uL of 1 mg/ml RNase A and incubate for 10 min at 37°C or room temp. If necessary, the RNase may be inactivated by Proteinase K digestion followed by phenol/chloroform extraction and alcohol precipitation.
DNA concentration is too low	If the DNA concentration is too low for some applications, it can be increased by alcohol precipitation. Add one-tenth volume of 5 M NaCl, mix, chill at least 30 min at -20°C or colder, then centrifuge for 20 min at 12,000 rpm or higher, preferably at 4°C. Thoroughly remove the supernatant fluid and resuspend the pellet in lower volume of DNA Elution Solution or other solvent.