艾科瑞生物
Accurate Biology

Instruction for Use

SteadyPure Soil DNA Extraction Kit

AG 21039
Version.V1E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This product is designed for DNA extraction from soil samples weighing less than 500 mg. This product utilizes a unique adsorbent that effectively removes common inhibitory factors, such as humic acids, which are widely present in soil. The kit also includes grinding beads to facilitate the suspension and lysis of soil samples. The purified DNA is of high integrity, purity, and stability, and is suitable for direct use in molecular biology applications, including PCR, qPCR, and NGS sequencing analysis.

2. Kit Information

Kit Name	Cat. No	Specification
SteadyPure Soil DNA Extraction Kit	AG 21039	50 rxns

3. Transportation and Storage

Storage	Store at Room Temperature
Transportation	Transport at Room Temperature

4. Kit Components

Buffer RS-3	20 ml x 2 pcs
Buffer LS-7	1.5 ml x 2 pcs
Buffer PA-3	13 ml
Buffer PS-2	10 ml
Buffer BS-6	60 ml
Buffer WA	25 ml
Buffer WB*	27 ml
Elution Buffer	10 ml
Grinding Beads	50 pcs
Soil DNA Mini Columns	50 sets
Collection tubes	50 pcs

*: Before the first use, add 63 mL of anhydrous ethanol to Buffer WB (the volume ratio of Buffer WB to anhydrous ethanol is 3:7). Mix thoroughly and label the bottle. Store at room temperature.

5. Not Provided Experimental Materials

Anhydrous Ethanol, 2 mL Centrifuge Tube, 5 mL Centrifuge Tube, 15 mL Centrifuge Tube, Water Bath.

6. Recommendation and Experimental Precautions

(1) During the experiment, strong denaturants may be encountered. Please wear appropriate laboratory clothing, safety goggles, mask, and gloves before starting the experiment.

Note: Waste liquids generated during the experiment must be collected in a designated waste container for proper disposal.

(2) If the buffer solutions Buffer LS-7, Buffer BS-6, or Buffer WA are accidentally spilled, immediately rinse with a large amount of water.

(3) For detailed information, please refer to the relevant MSDS (Material Safety Data Sheet) information.

(4) When stored at low temperatures, components Buffer LS-7 and Buffer BS-6 may form precipitates. Heat the mixture at 37°C until the precipitate dissolves, then mix thoroughly before use.

(5) When eluting DNA bound to the Soil DNA Mini Column, heat the Elution Buffer or sterile water to 50-65°C to enhance DNA elution efficiency.

(6) Before the first use of Buffer WB, add 63 mL of anhydrous ethanol (the volume ratio of Buffer WB to anhydrous ethanol is 3:7). Mix thoroughly and label the bottle. Store at room temperature.

(7) The reagent volumes for Buffer RS-3, Buffer PA-3, and Buffer PS-2 should not be altered. Experimental evidence has shown that increasing the amounts of these reagents does not improve DNA yield.

(8) Fresh experimental materials should be used whenever possible to ensure that DNA in the sample is not degraded.

(9) The sample size should not exceed the maximum starting amount (500 mg). Exceeding this limit may result in insufficient suspension of the sample, leading to incomplete lysis and negatively impacting DNA yield and purity.

(10) The humic acid content in different soil types varies. If humic acid removal is incomplete during extraction (generally, the supernatant in <Sample Processing Step 7> should be colorless or pale yellow; if the supernatant remains dark yellow or brown, it may indicate incomplete removal of humic acids), repeat <Sample Processing Step 7> to further remove humic acids and other impurities.

(11) The maximum loading volume for the Soil DNA Mini Column in this product is 750 µL. If the liquid volume exceeds the maximum loading capacity, add the mixture in multiple aliquots: load 750 µL of the mixture, centrifuge, discard the filtrate, then add the remaining mixture in the same manner. Repeat the steps.

(12) During the process, the Soil DNA Mini Column adsorption column should be handled vertically when being removed from the collection tube or 1.5 mL centrifuge tube (or placed into it) to avoid contamination by the column tip touching the tube wall.

(13) If the extracted DNA needs to be stored for an extended period, it is recommended to elute the DNA using Elution Buffer.

7. Protocol

Sample Processing Step

1. Weigh less than 500 mg of soil sample and transfer it into a centrifuge tube containing Grinding Beads.

2. Add 750 µL of Buffer RS-3 and 60 µL of Buffer LS-7 to the centrifuge tube. Vortex at high speed to thoroughly suspend and mix the sample, ensuring complete lysis.
Note: The soil sample must be fully suspended in the solution to avoid affecting DNA yield and purity. The volume of the soil sample may vary depending on soil type, even for the same mass. If the sample volume is too large, it may result in insufficient suspension, so it is recommended to reduce the starting sample volume, ensuring the soil sample is fully suspended in Buffer RS-3.

3. Centrifuge at 12,000 rpm for 3 minutes at room temperature. Transfer the supernatant to a new 2 mL centrifuge tube.
Note: The lower precipitate contains soil residues. When transferring the supernatant, avoid taking any of the lower precipitate to prevent contamination of the DNA purity.
Note: Aspirate as much supernatant as possible without disturbing the precipitate to maximize DNA yield.

4. Add 250 µL of Buffer PA-3 to the centrifuge tube. Vortex for 30 seconds to mix thoroughly, then place on ice and incubate for 5 minutes.

5. Centrifuge at 12,000 rpm for 1 minute at room temperature. Transfer the supernatant to a new 2 mL centrifuge tube.
Note: The lower precipitate contains humic acids and other impurities. Avoid transferring any of this precipitate when transferring the supernatant to ensure the purity of the DNA.
Note: Aspirate as much supernatant as possible without disturbing the precipitate to increase DNA yield.

6. Add 200 µL of Buffer PS-2 to the centrifuge tube. Vortex for 30 seconds to mix thoroughly, then place on ice and incubate for 5 minutes.

7. Centrifuge at 12,000 rpm for 1 minute at room temperature. Transfer the supernatant to a 5 mL or 15 mL centrifuge tube.
Note: The lower precipitate contains humic acids and other impurities. Avoid transferring any of the lower precipitate to ensure DNA purity.
Note: Aspirate as much supernatant as possible without disturbing the precipitate to maximize DNA yield.

8. Add 1200 µL of Buffer BS-6 to the centrifuge tube. Pipette to mix thoroughly.

Purification Steps

1. Transfer the entire mixture to the Soil DNA Mini Column and incubate at room temperature for 1 minute. Centrifuge at 12,000 rpm for 1 minute at room temperature, then discard the filtrate.
Note: The maximum sample volume for the Soil DNA Mini Column in this product is 750 µL. For this step, please add the mixture in aliquots of 750 µL each. After each addition, centrifuge and discard the filtrate, then add the remaining mixture and repeat this process.

2. Add 500 µL of Buffer WA to the Soil DNA Mini Column, centrifuge at 12,000 rpm for 1 minute at room temperature, then discard the filtrate.

3. Add 750 µL of Buffer WB to the Soil DNA Mini Column, centrifuge at 12,000 rpm for 1 minute at room temperature, then discard the filtrate.
Note: Ensure that the specified volume of anhydrous ethanol has been added to Buffer WB.

4. Repeat Purification Step 3 once more.

5. Place the Soil DNA Mini Column into a new 2.0 mL Collection Tube and centrifuge at 12,000 rpm for 2 minutes at room temperature. Discard the filtrate.

6. Place the Soil DNA Mini Column into a new 1.5 mL centrifuge tube. Add 50 µL to 100 µL of Elution Buffer or sterile water to the center of the membrane, incubate at room temperature for 2 minutes, then centrifuge at 12,000 rpm for 2 minutes at room temperature to elute the DNA. The obtained DNA can be used directly for subsequent analysis or stored at -20°C.
Note: Heating Elution Buffer or sterile water to 50 ~ 65°C before use can enhance the elution efficiency. To obtain a higher DNA yield, the elution liquid from Purification Step 6 can be transferred back to the Soil DNA Mini Column for a second elution.
Note: The amount of Elution Buffer or sterile water added can be adjusted based on the required DNA concentration. For higher DNA concentration, reduce the amount of Elution Buffer or sterile water (e.g., 30 ~ 50 µL).

AG 艾科瑞生物 Accurate Biology

Accurate Biotechnology (Hunan) Co., Ltd
Hunan Inspection Industrial Park, Bachelor Road,
Yuelu District, Changsha City, Hunan Province, China
service@agbio.com.cn
+86 400 767 6022
en.agbio.com.cn
Research Use Only