SIMGEN

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Sputum DNA Extraction Kit Instructions

Composition

Sputum DNA Extraction Kit Cat. No.	5 Preps 3502005	50 Preps 3502050
Spin Columns	5	50
2 ml Collection Tubes	5	50
Buffer SP	100 μl	1 ml
Proteinase K	120 μl	1.2 ml
Buffer DT	10 ml	50 ml×2
Buffer SD (concentrate)	6 ml	60 ml
Buffer WN (concentrate)	1 ml	10 ml
Buffer WB (concentrate)	1.5 ml	9.5 ml
Buffer TE	1.2 ml	12 ml
Instructions	1	1

Storage

1. Proteinase K and Buffer SP should be stored at -20°C.
2. Other reagents and items, if stored at room temperature (0~30°C), can maintain no significant change in performance within 2 years; If stored at 2~8°C, the validity period can be extended to more than 2 years (products stored at 2~8°C should be restored to room temperature before use).

Technical Support

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Introduction

This product is suitable for total DNA (including genomic DNA, mitochondrial DNA and possible bacterial and viral DNA) extraction from 1 ml liquefied sputum. After digestion by proteinase K, the DNA will be bound to the silica gel particles and Spin Columns, the degraded proteins and PCR inhibitors will be filtered out, and the DNA will be washed by Buffer WB and absolute ethanol and eluted with Buffer TE, which can be used in various molecular biology experiments.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol.
2. 1.5 ml centrifuge tube, 15 ml centrifuge tube and pipette and tips.
3. Disposable gloves, protective equipment and paper towels.
4. Microcentrifuge(s) (with rotors for 1.5 ml tubes, 2 ml tubes and 15 ml tubes).
5. Thermostat shaker, water bath (dry bath) and vortexer.

Preparation before use

1. If the centrifuge has a refrigeration function, set the temperature to 25°C.
2. Set the thermostat shaker temperature to 37°C. Set the water bath temperature to 60°C and incubate Buffer TE to 60°C.
3. Add absolute ethanol to Buffer SD, Buffer WN, and Buffer WB according to the instructions on the label of the reagent bottle and tick the box on the label to mark "Ethanol Added".

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Protocol

1. **Estimate the volume of sputum, add Buffer DT according to 1~1.5 times the volume of sputum, then add 15 µl Buffer SP, incubate at 37°C for 0.5~2 hours in the thermostat shaker, so that the sputum is fully liquefied.**

* The shaking time can be reduced or increased according to the viscosity of the sputum.

2. **Transfer 1 ml of liquefied sputum into a 15 ml centrifuge tube. Shake the reagent bottle containing Buffer SD vigorously to fully suspend the silica gel particles in the solution, add 2 ml Buffer SD, vortex for 15 sec to mix well.**

* If the liquefied sputum is jellylike (usually the state of yellow brown thick sputum after liquefaction, it will have great resistance when transfer with 1 ml tip), 0.3~0.5 ml of liquefied sputum should be transferred into 15 ml centrifuge tube, and Buffer DT should be added to dilute the liquefied sputum to 1 ml. The following operation steps remain unchanged.

* If excessive jellylike liquefied sputum is used, it may exceed the digestive capacity of subsequent proteinase K, and the excess DNA released will cause the silica gel particles to agglutinate into flocculent, resulting in lower recovery efficiency and poor purity of the final DNA.

* Ensure that absolute ethanol has been added to Buffer SD.

* The silica gel particles in Buffer SD must be fully suspended and added to the sputum, otherwise the sensitivity of subsequent tests will be affected.

3. **Centrifuge at 2000 rpm for 5 min. Discard 2.7 ml of the supernatant, retaining the precipitation of silica gel particles and part of the supernatant.**
4. **Add 20 µl of Proteinase K and vortex for 15 sec to mix well. Incubate at 60°C for 20 min.**
5. **Add 300 µl Buffer WN and pipette several times to mix well.**

* Ensure that absolute ethanol has been added to Buffer WN.

6. **Transfer the mixture from Step 5 (including silica gel particles) to a Spin Column (the Spin Column is placed in the 2 ml Collection Tube), close the lid and centrifuge at 12000 rpm for 30 sec.**

* There is DNA adsorbed in the silica gel particles, and all of it must be transferred to the Spin Column.

* Be careful not to get the mixture on the edge of the Spin Column nozzle to avoid disturb subsequent wash steps.

7. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 600 µl Buffer WB to the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**

* Ensure that absolute ethanol has been added to Buffer WB.

8. **Discard the filtrate, place the Spin Column back into the 2 ml Collection Tube, add 700 µl absolute ethanol to the Spin Column, close the lid and centrifuge at 12000 rpm for 30 sec.**
9. **Discard the filtrate, place the Spin Column back to the 2 ml Collection Tube and centrifuge at 14,000 rpm for 1 min.**

* If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.

* Do not omit this step, or the exacted nucleic acid maybe mixed with ethanol.

10. **Discard 2 ml Collection Tube, place the Spin Column in a clean 1.5 ml centrifuge tube, add 100-200 µl Buffer TE incubated to 60°C into the Spin Column, close the lid, incubate at room temperature for 2 min, and centrifuge at 12000 rpm for 30 sec.**

* Sticky sputum contains high DNA content. If it is a sputum sample, please elute DNA with at least 200 µl Buffer TE, otherwise the DNA concentration is too high, which may affect the subsequent DNA quantification and detection.

* If the centrifuge does not have a leak-proof cover, change the centrifuge condition to 8000 rpm for 1 min to avoid damage to the centrifuge due to the removal of the 1.5 ml centrifuge tube lid.

11. **Discard the Spin Column, and the eluted DNA can be immediately used in various molecular biology experiments or stored at -20°C for later use.**

* If the eluted DNA contains a small amount of silica gel particles, close the lid after discarding the Spin Column, centrifuge at the full speed for 1 min, transfer the DNA supernatant and use directly or transfer the DNA supernatant to another clean 1.5 ml centrifuge tube and store at -20°C for later use.