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Quick DNA Lyse & Det PCR Kit Instructions

Composition

Quick DNA Lyse & Det PCR Kit Cat. No.	5 Preps 4005005	50 Preps 4005050	200 Preps 4005200
Lysis Buffer	1.2 ml	11 ml	11 ml×4
Proteinase K	60 µl	600 µl	1.2 ml×2
2×PCR Mix	60 µl	600 µl	1.2 ml×2
ddH_2O	50 µl	500 µl	1 ml×2
Grinding Rod	5	5	5
Instructions	1	1	1

Storage

The reagents and items store at -20℃, the expiration date is more than 2 years. If used frequently, it is recommended to store at 2~8℃, the expiration date is 6 months.

Technical Support

R&D Department, Hangzhou Simgen Biotechnology Co., Ltd. E-mail: technical@simgen.cn, Tel: 400-0099-857.

Introduction

This kit contains all the reagents for quick DNA extraction and PCR amplification, allowing one-step extraction of total DNA from plant, seed, bacterial, and animal tissue samples for PCR amplification in less than 20 min. The whole extraction process does not require organic solvent extraction and ethanol precipitation, which is simple, fast, and stable and reliable in quality.

The 2× PCR Mix provided with this kit is an optimized two-fold concentration PCR master mix with Taq Plus DNA polymerase, PCR enhancer, and protein stabilizer synergistically improve PCR efficiency and sensitivity, making it ideal for low-copy template amplification, especially for high-throughput screening.

Equipment And Reagents to Be Supplied by Users

1. 1.5 ml centrifuge tubes, pipette tips, disposable gloves, and protective equipment
2. Microcentrifuge (s) (with rotors for 1.5 ml and 2 ml centrifuge tubes)
3. Vortexer, water bath, or dry bath

Preparation before use

1. If the centrifuge has refrigeration, set the temperature to 25℃.
2. Set the water bath or dry bath temperature to 56℃ and 95℃.
3. Samples collected:

   Plant material: weigh about 10 mg of tissue and put it into a centrifuge tube, rotate and squeeze the tissue with a Grinding Rod to make it into a homogenized paste (if you need more Grinding Rods, you can call 400-0099-857 to inquire about purchasing).

   Animal material: chop the tissue into a homogenate with a scalpel, weigh about 10 mg of tissue homogenate and put it into a centrifuge tube.

   Bacteria: collect 200-800 µl cultured bacterial, centrifuge at 12000 rpm for 30 sec, discard the culture medium.

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Protocol

1. **Add 200 µl Lysis Buffer and vortex until tissue homogenate or bacteria are completely suspended and dispersed.**
2. **Add 10 µl Proteinase K, vortex to mix well, and incubate at 56℃ for 10 min.**
3. **Spin down the liquid on the lid to the bottom of the tube, and then incubate at 95℃ for 5 min.**

* Do not omit the step of spin down, otherwise the residual proteinase K on the lid is not inactivated at 95℃ and may affect subsequent PCR amplification.

4. **Centrifuge at the full speed (≥13,000 rpm) for 5 min.**
5. **Transfer 150 µl supernatant to a clean 1.5 ml centrifuge tube, use it as a template directly for PCR amplification or store at -20℃ for later use.**
6. **PCR amplification：**

2×PCR Mix	10 µl
Forward primer (10 µM)	0.5 µl
Reverse primer (10 µM)	0.5 µl
template	2 µl
ddH_2O	7 µl
Total	20 µl

7. **Flick the PCR reaction tube to mix well and spin down.**
8. **Example of PCR reaction cycle setup**

94℃ 3 min
94℃ 30 sec
※55℃ 30 sec } 35 Cycles
§72℃ 1 min
72℃ 5 min

*The actual optimal annealing temperature shall prevail.

§ Calculated at 1 kb/min.

For amplification of fragments of interest below 300 bp, a two-step amplification method can be used to save amplification time:

94℃ 3 min
94℃ 20 sec
60℃ 1 min } 35 Cycles
72℃ 5 min

9. **Results: 5-10 µl the amplified product was directly detected by agarose electrophoresis.**

Relationship between agarose gel concentration and the optimal resolution range of linear DNA:

Agarose	Optimal DNA resolution range (bp)
0.5%	1,000~30,000
0.7%	800~12,000
1.0%	500~10,000
1.2%	400~7,000
1.5%	200~3,000
2.0%	50~2,000