

TIANcombi DNA Lyse & Det PCR Kit

Fastest DNA Extraction from Plant,
Bacteria and Tissue Samples Followed by
PCR

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medicine, clinical treatment, food or cosmetics.

TIANcombi DNA Lyse & Det PCR Kit

Cat. No. 4992527/4992528

Kit Contents

| Contents | 4992527 50 preps | 4992528 200 preps |
|----------------------|---------------------|----------------------|
| Buffer B1 | 6 ml | 24 ml |
| Buffer B2 | 6 ml | 24 ml |
| 2× Det PCR MasterMix | 500 µl | 2× 1 ml |
| Grinding Pestles | 10 | 20 |
| Handbook | 1 | 1 |

Compatible device

TGrinder (Cat. No. OSE-Y50)

Storage

Buffer B1 and Buffer B2 can be stored at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. 2× Det PCR MasterMix could be stored at -20°C for long periods, and repeated freezing-thawing will not affect its activity. For frequent use of 2× Det PCR MasterMix, please store at 4°C.

Introduction

TIANcombi DNA Lyse & Det PCR Kit uses special buffer system for fast one-step DNA extraction and PCR from a wide range of starting materials, including plant tissues, seeds, animal tissues, blood, yeast and bacteria. The kit contains both the solution for DNA extraction and the PCR amplification reagent. The whole extraction process will not include protein, RNA and secondary metabolite removal step, as well as phenol extraction and ethanol precipitation. Grinding in liquid nitrogen is also not required.

The 2× Det PCR MasterMix could amplify raw samples with high compatibility, high efficiency and specificity without removing impurities such as proteins. This reagent contains *Taq* DNA polymerase, dNTPs, MgCl₂, buffer, PCR enhancers and stabilizers, which is fast and simple to use, and especially qualified for high-throughput screening.

Features

Simple and fast: DNA from different tissues can be extracted in 5 min without the need for liquid nitrogen grinding.

Wide applications: Applicable for plant leaves, seeds, animal tissues, blood samples (e.g. fresh blood, anti-coagulation blood, blood clots, dried blood spots), yeast and bacteria, etc.

Good compatibility: The PCR reagents in this kit are suitable for the amplification of DNA extracted from various sample sources.

Gene detection: Especially suitable for large-scale gene detection.

Important Notes Before Starting

1. Avoid repeated freezing and thawing of the sample, otherwise the extracted DNA fragments will be smaller and the extraction yield will be decreased.
2. For phenols-rich samples like cotton leaves, the samples amount should not be over 0.4 mg, or it will reduce PCR reaction efficiency.
3. Buffer B1 and Buffer B2 should be stored at room temperature (18-25°C). If precipitation forms at low temperature (-20°C or 4°C), redissolve the buffer in 37°C water bath and use it after mixing the solution.
4. 2× Det PCR MasterMix provided in this kit is a 2× stock solution. Template, primers and sterilized water should be added to make up to a 1× solution before use.

Protocol

1. For first use, please check if there is precipitate formed in the two bottles of buffer. If there is, please put the buffer at room temperature or 37°C water bath until the precipitation is dissolved. The dissolved buffer should be stored at room temperature.
2. Put small amount (take table 1 for reference) of samples to 1.5 ml centrifuge tube, add 100 µl Buffer B1, and make sure that Buffer B1 could completely cover the sample.
3. Grind samples with a grinding pestle.

Note: For blood or bacteria samples which are difficult to discriminate whether have been fully grinded, please grind for 30 sec with grinding pestle. And bacteria samples should be centrifuged to collect the cell pellet. For plant seeds, skin, connective tissue and etc., please grind it to be muddy with grinding pestle (TGrinder (Cat. no. OSE-Y50) will be more convenient to use).

4. Add 100 µl Buffer B2, vortex to mix, then centrifuge at 12,000 rpm (~13,400 x g) for 2 min.
5. Pipet 100 µl supernatant to a clean 1.5 ml centrifuge tube to be used as template.
6. Set up the PCR amplification reaction.

Reaction system

| Components | Volume |
|-----------------------|-------------|
| 2× Det PCR MasterMix | 10.0 µl |
| Forward Primer (10µM) | 0.5 µl |
| Reverse Primer (10µM) | 0.5 µl |
| Template DNA | 1.0 µl |
| ddH ₂ O | up to 20 µl |

PCR cycle set-up:

| | | |
|------|--------|-------------|
| 94°C | 3 min | } 35 cycles |
| 94°C | 30 sec | |
| 55°C | 30 sec | |
| 72°C | 1 min | |
| 72°C | 5 min | |

Result Detection

Take 5-10 μ l reaction products for agarose electrophoresis detection.

Note: the example provided is for your reference. As the template and primers differs, the real reaction condition should be modified accordingly.

Appendix Table 1

| Samples | Volume |
|----------------|---|
| Plant leaves | 1-5 mg |
| Plant seeds | 1-5 mg (without seed peels) |
| Animal tissues | 1-5 mg |
| Bacteria | Cell pellet collected from 0.2-0.5 ml bacteria liquid |
| Yeast | Cell pellet collected from 0.2-0.5 ml yeast liquid |
| Blood | 20 μ l |