Instruction manual

CellEase® Plant Cat#BIC-BCR11-00002

1. Introduction

CellEase® Plant is the reagent kit for extraction of genomic DNA from plant tissue rapidly and efficiently. You can prepare PCR (Polymerase Chain Reaction) grade template DNA with simple protocol (mix with reagent and test samples and then incubate without any purification steps).

2. Contents

Reagent A : 750µl
Reagent B : 750µl
Reagent C : 750µl
Instruction manual : 1
Volume : 50 reactions
Store at : 4C
Please store the Reagent C at -20C after opening the tube.

3. Principle

The reagent A disrupts the cells and stabilizes the genomic DNA from the samples. The reagent B degrades the cell extracts quickly. You can prepare the DNA from the cells efficiently in short time. Furthermore reagent C inactivates the PCR inhibitors from the cell debris. These reagents don't have any inhibitor for PCR. Thus, you can use the DNA samples with CellEase® Plant directly to PCR. These CellEase® Plant reagents don't include any toxic or harmful chemical compounds.

4. Use

Plant tissue, etc.

5. Preparation

Prepare the CellEase® mixture before use. Mix well the reagent A and B (1:1). The mixture is not able to store for long time.

Table 1. Preparation of CellEase® mixture

Number of samples	Reagent A (µl)	Reagent B (µl)
1	15	15
5	75	75
10	150	150
25	375	375
50	750	750

6. Standard protocol (DNA template for PCR)

1) Cut samples into appropriate size and put in the micro-tube (The optimal sample amount will vary with the type of samples).
2) Add 30µl of CellEase® mixture into the samples and homogenize the sample tissue.
3) Incubate the samples at 72C for 6 minutes.
4) Then, continually incubate the samples at 94C for 3 minutes.
4) Add 15µl of regent C to the test samples and stir them gently.
5) Take appropriate amount (2～10µl) of extracts and use as a template DNA of PCR.

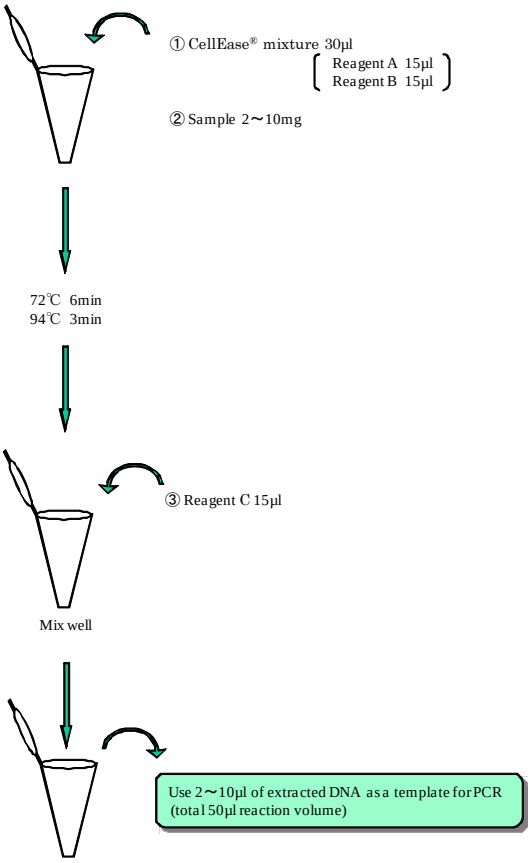


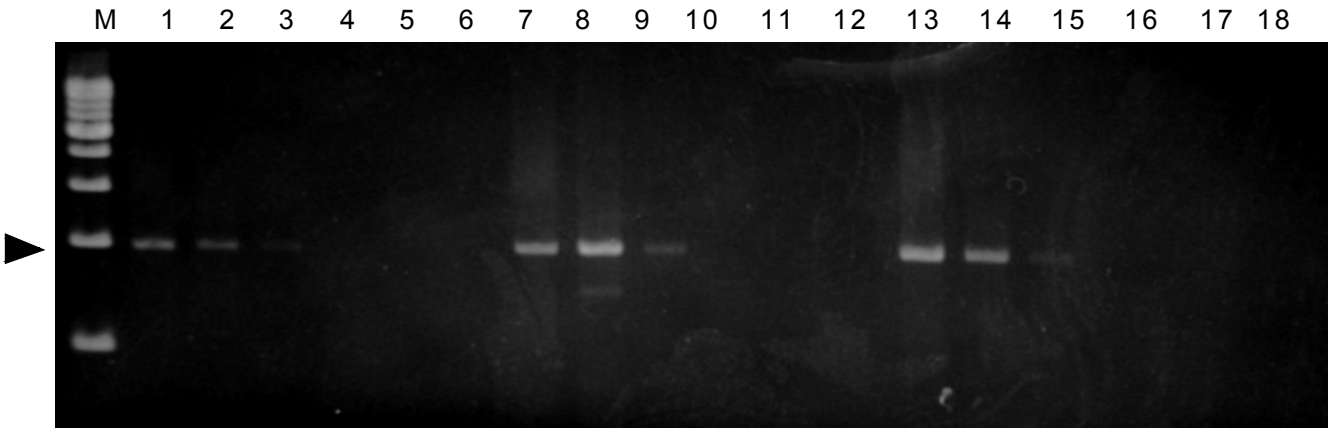
Fig. 1. The Schematic diagram for the extraction of DNA

5. Caution

1) You can adjust the volume of CellEase® mixture as amount of sample volume.
2) If it isn't enough DNA extracts with standard protocol. You can use a little longer incubation time (up to 60min) on the step of 72C.
3) The rate of the mixture of reagent A and B.
4) The DNA extracts should be added less than 20% of the total volume of PCR.
5) The DNA extracts can be kept at 4C for a few days. Alternatively it would be stored at -20C until use.
6) CellEase® Plant is for research use only. Please don't use for the other purpose.

COSMO BIO CO., LTD.
Inspiration for Life Science

DNA extraction from tomato leaf



M Marker（500bp ladder）

1	+ CellEase, Without dilution	Add 5µl to PCR
2	+ CellEase, ×10 dilution	Add 5µl to PCR
3	+ CellEase, × 100 dilution	Add 5µl to PCR
4	- CellEase, Without dilution	Add 5µl to PCR
5	- CellEase, ×10 dilution	Add 5µl to PCR
6	- CellEase, × 100 dilution	Add 5µl to PCR
7	+ CellEase, Without dilution	Add 6µl to PCR
8	+ CellEase, ×10 dilution	Add 6µl to PCR
9	+ CellEase, × 100 dilution	Add 6µl to PCR
10	- CellEase, Without dilution	Add 6µl to PCR
11	- CellEase, ×10 dilution	Add 6µl to PCR
12	- CellEase, × 100 dilution	Add 6µl to PCR
13	+ CellEase, Without dilution	Add 7µl to PCR
14	+ CellEase, ×10 dilution	Add 7µl to PCR
15	+ CellEase, × 100 dilution	Add 7µl to PCR
16	- CellEase, Without dilution	Add 7µl to PCR
17	- CellEase, ×10 dilution	Add 7µl to PCR
18	- CellEase, × 100 dilution	Add 7µl to PCR

※ The DNA extract was diluted by distilled water respectively and apply to PCR.
Primer: A part of heat shock protein gene (Hsc 70, 1kbp length) from Tomato (*Lycopersicon esculentum*)

Protocol of CellEase Plant

1) Cut 2×2mm of the leaf and put it in the micro test tube (ordinary use 0.2ml or 0.5ml tubes for PCR).
2) Mix the reagent A and B into microtube(15 µl of reagent A, 15 µl of reagent B).
3) Add 30µl of the mixture to the sample and homogenize the leaf tissue.
4) Incubate at 72C for 6 minutes. Then incubate at 94Cfor 3 minutes.
5) Add 15µl of the reagent C to the test sample.
6) Transfer 5-7µl of extracts to PCR reaction mixture and amplify the target DNA fragment.

PCR reagent

5～7	ul	Test sample
5.0	ul	×10 buffer（+Mg^{2+}）
5.0	ul	dNTPs
1.0	ul	Forward Primer (10pmol/ul)
1.0	ul	Reverse Primer (10pmol/ul)
0.5	ul	Ex Taq DNA polymerase (5 U/ul, Takara, Japan)

Fill up to 50ul by distilled water

PCR Cycle

94℃	1min
94℃	30sec
55℃	30sec
72℃	60sec
72℃	4min

35 Cycles

※We can provide application data for the other kind of samples. Please don't hesitate to contact us.
※CellEase® is the registered trademark of Biocosm Inc.

For research use only. Not for clinical diagnosis.

Manufactured by *Biocosm Inc.*

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