GENEzol™ DNA Reagent Plant

Catalogue Numbers	Quantity
GR100	100 ml
GR200	200 ml

Geneaid

CERTIFICATE NO. QAIC/TW/50077
ISO 9001:2008 QMS

Introduction

GENEzol™ DNA Reagent Plant provides a quick and easy 3 step CTAB and chloroform based method to isolate total DNA (including genomic, mitochondrial and chloroplast DNA) from a variety of plant species (including algae and cyanobacteria). This unique reagent is able to lyse most common plant samples and plant samples with high a polysaccharide content. The extracted DNA is suitable for routine PCR screening, Real-Time PCR, Southern Blotting, Mapping and RFLP. Phenol extraction is not required and the entire procedure can be completed within 50 minutes.

Quality Control

GENEzol™ DNA Reagent Plant is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system. 50 mg of fresh *Arabidopsis* leaves are initially ground in GENEzol™ DNA Reagent Plant. A 15 µl aliquot of extracted genomic DNA from a 100 µl eluate is analyzed by electrophoresis on a 1% agarose gel.

Advantages

- High molecular weight genomic DNA extraction from a variety of plant species
- Sample: up to 1 g of fresh plant tissue and up to 0.5 g of dry plant tissue
- Scalable, simple and gentle CTAB and chloroform based DNA precipitation method
- Cost effective

Applications

PCR, Real-Time PCR, Southern Blotting, Mapping and RFLP

Caution

GENEzol™ DNA Reagent Plant contains irritants. During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.

Additional Requirements

mortar and pestle, 1.5 ml microcentrifuge tubes or 15 ml centrifuge tubes, absolute ethanol for preparing 70% ethanol in water, chloroform, isopropanol, TE buffer or ddH_2O

Components and Storage

Item	Volume	Product	Shipping	Storage
GENEzol™ DNA Reagent Plant	4 ml	GR004	room temperature	dry at room temperature (15-25ºC)
	100 ml	GR100		
	200 ml	GR200		
RNase A (50 mg/ml)	N/A	GR004	room temperature	4ºC for extended periods
	50 µl	GR100		
	100 µl	GR200		

Scaling Large Sample Volumes

Plant tissue	100 mg	500 mg
Tube size	1.5 ml	15 ml
GENEzol™ DNA Reagent Plant	1 ml	5 ml
RNase A (50 mg/ml)	0.5 µl	2.5 µl
Chloroform	600 µl	3 ml
Isopropanol	800 µl	4 ml
70% ethanol	1 ml	5 ml

Fast PCR Grade DNA Protocol Procedure

Please read the entire instruction manual prior to starting the Protocol Procedure.

1. Plant Tissue Homogenization

Cut off **50 mg of fresh plant tissue or 25 mg of dry plant tissue**. Freeze the sample with liquid nitrogen (some plant samples can be disrupted without liquid nitrogen). Grind the sample to a fine powder using a mortar and pestle.

2. Lysis

Add **800 µl of GENEzol™ Reagent Plant and 0.5 µl of RNase A** to the sample in the mortar. Continue grinding the sample until it is completely dissolved. Transfer the sample lysate to a 1.5 ml microcentrifuge tube. Incubate the sample lysate at 65ºC for 15 minutes then centrifuge at 14-16,000 x g for 3 minutes. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

3. DNA Precipitation

Add **600 µl of isopropanol** to the supernatant in the 1.5 ml microcentrifuge tube. Mix the sample by gently inverting 20 times then let stand for 5 minutes at room temperature. Centrifuge at 14-16,000 x g for 15 minutes to form a tight, well formed DNA pellet. Carefully remove the supernatant then add **1 ml of 70% ethanol** to the DNA pellet and wash by gently inverting 20 times. Centrifuge at 14-16,000 x g for 3 minutes.

Carefully remove the supernatant then air-dry the DNA pellet for 10-15 minutes at room temperature.

NOTE: DO NOT dry the DNA pellet by vacuum centrifuge and avoid over drying the DNA pellet.

Add **50-100 µl of TE buffer or ddH2O** to the DNA pellet then incubate at 65ºC for 5 minutes to dissolve the DNA.

NOTE: Occasionaly tapping the bottom of the tube during incubation will promote DNA rehydration.

Centrifuge at 14-16,000 x g for 1 minute then transfer the supernatant (containing the purified DNA) to a clean 1.5 ml microcentrifuge tube. The purified DNA is ready for routine PCR assays.

High Purity and High Yield DNA Protocol Procedure

Please read the entire manual prior to starting the procedure.

1. Plant Tissue Homogenization

Cut off **100 mg of fresh plant tissue or 50 mg of dry plant tissue**. Freeze the sample with liquid nitrogen (some plant samples can be disrupted without liquid nitrogen). Grind the sample to a fine powder using a mortar and pestle.

2. Lysis

Add **1 ml of GENEzol™ Reagent Plant and 0.5 µl of RNase A** to the sample in the mortar.

NOTE: If using more than 100 mg of plant tissue, scale GENEzol™ Reagent Plant proportionately (see table on page 3).

Continue grinding the sample until it is completely dissolved then transfer the sample lysate to a 1.5 ml microcentrifuge tube.

NOTE: If using more than 100 mg of plant tissue, transfer the sample lysate to a 15 ml centrifuge tube.

Incubate the sample lysate at 65ºC for 30 minutes then centrifuge at 14-16,000 x g for 5 minutes. Transfer the supernatant to a new 1.5 ml microcentrifuge tube or a new 15 ml centrifuge tube for larger sample sizes.

3. DNA Extraction

Standard Samples:

Add **600 µl of chloroform** to the supernatant.

NOTE: Scale the chloroform proportionately if using larger sample sizes (see table on page 3).

Shake the tube vigorously then centrifuge at 14-16,000 x g for 5 minutes. Carefully transfer the colorless aqueous phase (upper layer) to a new 1.5 ml microcentrifuge tube or a new 15 ml centrifuge tube for larger sample sizes.

High Polysaccharide Samples:

Add a **1/10 volume of GENEzol™ Reagent Plant and 600 µl of chloroform** to the supernatant from Step 2.

NOTE: Scale GENEzol™ Reagent Plant and chloroform proportionately if using larger sample sizes (see table on page 1).

Shake the tube vigorously then centrifuge at 14-16,000 x g for 5 minutes. Carefully transfer the colorless aqueous phase (upper layer) to a new 1.5 ml microcentrifuge tube or a new 15 ml centrifuge tube for larger sample sizes.

4. DNA Precipitation

Add **800 µl of isopropanol** to the 1.5 ml microcentrifuge tube containing the upper layer from step 3.

NOTE: Scale isopropanol proportionately if using larger sample sizes (see table below).

Mix the sample by gently inverting 20 times then let stand for 5 minutes at room temperature.

NOTE: DNA precipitation can be increased with extended standing time.

Centrifuge at 14-16,000 x g for 20 minutes to form a tight, well formed DNA pellet. Carefully remove the supernatant then add 1 ml of 70% ethanol to the DNA pellet and wash by gently inverting 20 times. Centrifuge at 14-16,000 x g for 3 minutes. Carefully remove the supernatant then air-dry the DNA pellet for 10-15 minutes at room temperature.

NOTE: DO NOT dry the DNA pellet by vacuum centrifuge and avoid over drying the DNA pellet.

Add 50-100 µl of TE buffer or ddH_2O to the DNA pellet then incubate at 65ºC for 10 minutes to dissolve the DNA.

NOTE: Occasionaly tapping the bottom of the tube during incubation will promote DNA rehydration.

Centrifuge at 14-16,000 x g for 1 minute then transfer the supernatant (containing the purified DNA) to a clean 1.5 ml microcentrifuge tube. The purified DNA is ready for downstream applications.