Presto™ Rapid Extract PCR kit

For research use only

Store ZEROprep™ DNA Reagent at room temperature

Store Geneaid™ Hot Start Taq Master Mix with Dye at 2-8 °C

Introduction

Presto™ Rapid Extract PCR kit consists of ZEROprep™ DNA Reagent and Geneaid™ Hot Start Taq Master Mix with Dye. ZEROprep™ DNA Reagent is designed for efficient release of DNA from variety of samples for direct use in PCR reactions without purification. In addition, Geneaid™ Hot Start Taq PCR Master Mix with Dye includes all of the components necessary to perform PCR. Add primers, template DNA and sterile water to complete the PCR reaction mix in routine PCR assays. Simply place the samples in the ZEROprep™ DNA reagent, only 10 minutes incubation and transfer the sample lysate as DNA template to Geneaid™ Hot Start Taq Master Mix with Dye for PCR assay. After PCR reaction, the PCR mixture could be conveniently loaded on the agarose gel without mix with loading dye in advance.

Quality Control

Presto™ Rapid Extract PCR kit is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system. DNA from a 1 mg tissue sample is lysed in ZEROprep™ DNA Reagent. A 2 µl aliquot of lysate is added directly into a 25 µl PCR mix using Geneaid™ Hot Start Taq PCR Master Mix with Dye.

Kit Contents

Component	RPCR004	RPCR100	RPCR500
ZEROprep™ DNA Reagent	**500 µl**	**10 ml**	**50 ml**
Geneaid™ Hot Start Taq Master Mix with Dye	**50 µl**	**1.25 ml**	**1.25 ml x 5**

Applications

Direct use of DNA in PCR reactions, multiplex PCR

Caution

During operation, always wear a lab coat, disposable gloves, protective goggles or (anti-fog) procedure mask.

ZEROprep™ DNA Reagent Protocol Procedure

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

Sample	Procedure
Tissue	1. **Transfer 50 µl of ZEROprep™ DNA Reagent and 1 mg of tissue to a 1.5 ml microcentrifuge tube.** 2. Incubate for 15 minutes at room temperature or 5-15 minutes at 80ºC. 3. Mix by vortex then transfer a **2 µl aliquot** to a **25 µl PCR mix using 2X Hot Start Taq Master Mix with Dye**.
Plant Tissue[1]	1. **Transfer 200 µl of ZEROprep™ DNA Reagent and 5-25 mg of tissue to a 1.5 ml microcentrifuge tube.** 2. Incubate for 15 minutes at room temperature or 5-15 minutes at 80ºC. 3. Mix by vortex then transfer a **2 µl aliquot** to a **25 µl PCR mix using 2X Hot Start Taq Master Mix with Dye**.
Whole Blood, plasma, serum	1. **Transfer 100 µl of ZEROprep™ DNA Reagent and 5-10 µl of fluid sample to a 1.5 ml microcentrifuge tube.** 2. Incubate for 15 minutes at room temperature. 3. Mix by vortex then transfer a **2 µl aliquot** to a **25 µl PCR mix using 2X Hot Start Taq Master Mix with Dye**.
Saliva	1. **Transfer 100 µl of ZEROprep™ DNA Reagent and 10 µl of saliva to a 1.5 ml microcentrifuge tube.** 2. Incubate for 15 minutes at room temperature or 10 minutes at 80ºC. 3. Mix by vortex then transfer a **2 µl aliquot** to a **25 µl PCR mix using 2X Hot Start Taq Master Mix with Dye**.
Bacteria[2]	1. **Transfer 100 µl of ZEROprep™ DNA Reagent and 1-5 µl of bacteria culture to a 1.5 ml microcentrifuge tube.** 2. Incubate for 15 minutes at room temperature or 10-15 minutes at 80-90ºC. 3. Mix by vortex then transfer a **2 µl aliquot** to a **25 µl PCR mix using 2X Hot Start Taq Master Mix with Dye**.

NOTE:

[1] For plant species with high levels of polysaccharide inhibitors, increase the tissue amount by 2-3 times per volume of ZEROprep™ DNA Reagent. Plant tissue homogenization using a bead beating instrument or pestle and mortar with liquid nitrogen will facilitate DNA release.

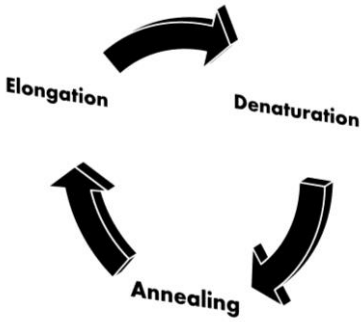
[2] *E.coli* can be efficiently lysed in ZEROprep™ DNA Reagent for 15 minutes at room temperature. However, to efficiently disrupt the bacteria cell wall of gram (+) bacteria, 3 hour incubation at room temperature or 10-15 minutes at 80ºC is required.

Geneaid™ Hot Start Taq PCR Master Mix with Dye Protocol

Reaction set up table

Component	For 25 µl	Final Conc.
Forward primer (10 µM)	0.25-2.5 µl	0.1-1.0 µM
Reverse primer (10 µM)	0.25-2.5 µl	0.1-1.0 µM
Sample lysate (DNA template)	1-2 µl	< 250 ng
Geneaid™ Hot Start Taq PCR Master Mix with Dye	12.5 µl	1X
Nuclease-free H_2O	Up to 25 µl	

General Geneaid™ Hot Start Taq PCR Master Mix with Dye Thermal Cycling Program

<table>
<tr><td>Initial Denaturation</td><td colspan="2">• 95ºC
• 1-5 minutes. (An initial denaturation of 1 min at 95°C is sufficient for most amplicons. For difficult templates, a longer denaturation of 2-3 min at 95°C is recommended. For colony PCR, an initial 5 min denaturation at 95°C is recommended.)</td></tr>
<tr><td>Step 2 Denaturation</td><td>• 95ºC
• 15-30 seconds.</td><td rowspan="3">25-35 Cycles
 
</td></tr>
<tr><td>Step 3 Annealing</td><td>• 50ºC-65ºC
• 30-60 seconds.
• Optimal annealing temperature is dependent on the Tm value of the primers and the reaction condition.</td></tr>
<tr><td>Step 4 Elongation</td><td>• 72ºC
• 1-5 minutes.
• For 1 Kb of PCR product, elongate at 72ºC for 1 minute. Adjust elongation time based on product size (1 Kb/minute) for PCR products larger than 2 Kb.)</td></tr>
<tr><td>Step 6 Final Elongation</td><td colspan="2">• 72ºC
• 7 minutes.</td></tr>
<tr><td>Step 7 Cooling</td><td colspan="2">• 4ºC</td></tr>
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