1560250-2/23

GenCatch™ Plant Genomic DNA Miniprep Kit

User's Guide for

Plant Genomic DNA Purification

For Research Use Only

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1 Quick Start Procedure

For Experienced Users Only.

First time users are strongly recommended to read through the detailed instruction protocol in section 4.

Before you start:

Add proper volume of 98-100% ethanol to WS Buffer and resuspend RNase A in proper volume of ddH2O according to page 4 of this instruction. Preheat elution buffer (H2O or TE) to 65°C for DNA elution.

Sample grind:	**Grind Sample**
Sample Lysis:	**+400 µl PX1, +4 µl RNase A Vortex, 65°C 10 min 130 µl PX2**
Clear lysate:	**Clear lysate using shearing tube**
Prepare for column Binding:	**+ 0.5 vol. PX3 and 1 vol. of 98%-100% ethanol, Mix**
Bind to Column:	**Apply to Maxi Column and centrifuge**
Wash with:	**700 µl WS, x2**
Remove Buffer:	**Centrifuge**
Elute DNA in:	**200 µl 65°C TE or ddH2O**

Downstream application, or store DNA at -20°C.

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2 Overview

GenCatch™ Plant Genomic DNA Miniprep Kit is designed for the extraction of high molecular weight genomic DNA from 100 mg plants samples or $1x10^8$ cells. This system employs silica membrane technology. Specific binding and subsequent efficient elution of gDNA from silica membrane can be achieved by simple centrifugation steps without phenol / chloroform.

Genomic DNA purified is free from contaminants and enzyme inhibitors, typical A260 / A280 ratio is around 1.8-2.0. The gDNA extracted has a MW distribution between 10-60kb with predominant around 30-kb, and is suitable for applications below.

Downstream Applications:

- PCR and qPCR
- Restriction enzyme digestion
- Labeling
- Ligation
- RAPD and RFLP
- Hybridization

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Product Contents

GenCatch™ Plant Genomic DNA Miniprep Kit contains sufficient reagents for 50 (Cat. No. 1560050) or 250 (Cat. No. 1560250) plant genomic DNA extraction applications.

Cat. No.	1560050	1560250
	50 preps	250 preps
PX1 Buffer	24 ml	120 ml
PX2 Buffer	8 ml	40 ml
PX3 Buffer	18 ml	90 ml
RNase A	20 mg	110 mg
WS Buffer	15 ml	45 ml x2
Plant Genomic DNA Maxi Column	50	250
Shearing Tube	50	250
Collection Tube	100	500

For Cat. No. 1560050 (50 preps)
Add 60 ml of ethanol (98-100 %) to the WS Buffer bottle when first open the bottle.
Add 200 µl of ddH2O to the RNase A powder tube, vortex to dissolve and store at 4°C.

For Cat. No. 1560250 (250 preps)
Add 180 ml of ethanol (98-100 %) to the WS Buffer bottle when first open the bottle.
Add 1100 µl of ddH2O to the RNase A powder tube, vortex to dissolve and store at 4°C.

Storage Conditions:
Store at room temperature

GenCatch™ Plant Genomic DNA Miniprep Kit is shipped and should be stored at ambient temperature up to 24 months.
If precipitate forms in any buffer, warm up at 37°C to redissolve.
GenCatch™ spin columns should be kept sealed in the zip lock bag provided during storage and away from any heating source.

4 Protocol

First time users are strongly recommended to read through this detailed protocol instruction.
For technical support please reach us at support@epochlifescience.com

Important Notes:

All centrifugation should be done at room temperature with a swing-bucket centrifuge.
Preheat a water bath to 65°C.
Preheat TE or ddH2O to 65°C for DNA elution.
PX1 buffer may become cloudy during storage. This does not affect the procedure and efficiency.
PX1 Buffer may form a precipitate, warm at 65°C to redissolve.

For Cat. No. 1560050 (50 preps)
Add 60 ml of ethanol (98-100 %) to the WS Buffer bottle when first open the bottle.
Add 200 µl of ddH2O to the RNase A powder tube, vortex to dissolve and store at 4°C.

For Cat. No. 1560250 (250 preps)
Add 180 ml of ethanol (98-100 %) to the WS Buffer bottle when first open the bottle.
Add 1100 µl of ddH2O to the RNase A powder tube, vortex to dissolve and store at 4°C.

1. Grind 100 mg (or less) plant sample under liquid nitrogen to a fine powder and transfer to a new tube.
   Do not allow the sample to thaw, and continue immediately to step 2.

2. Add 400 µl of PX1 Buffer and 4 µl of RNase A solution (100 mg/ml) to the tissue powder and vortex vigorously, then incubate the mixture at 65°C for 10 minutes.
   Do not mix PX1 Buffer and RNase A prior to use. Invert 2-3 times during 65°C incubation.

3. Add 130 µl of PX2 Buffer to the lysate, vortex, and incubate on ice for 5 minutes.

4. Apply lysate to the Shearing Tube sitting in a Collection Tube and centrifuge at full speed for 2 minutes. Transfer flow-through sample from the Collection Tube to a new tube (not provided).
   Avoid pipetting any debris or pellet in the collection tube.

5. Add 0.5 volume of PX3 Buffer and 1 volume of 98-100% ethanol to the clear lysate and mix by inverting the tube 3-5 times.
   For example: If 450 µl clear lysate collected, add 225 µl PX3 Buffer and 450 µl ethanol.

6. Load the ethanol added sample (including any precipitate) from step 5 to a GenCatch™ mini spin Column sitting in a Collection Tube, close the cap, centrifuge at full speed for 1 minutes, and discard the filtrate.
   If the solution remains above the membrane, centrifuge again.

7. Repeat step 6 for rest of the sample.

8. Wash the column twice with 700 µl of WS Buffer by centrifuging at full speed for 60 seconds and discard the filtrate.
   Add ethanol (98-100%) to the WS Buffer bottle when opened.

9. Centrifuge at full speed for 2 minutes to remove traces of WS Buffer.

10. Transfer the column to a new 1.5 ml tube (not provided), add 200 µl of 65°C TE or ddH2O. Stand the column for 5 minutes and centrifuge at full speed for 2 minute to elute DNA.

11. Store DNA at -20°C.