Genomic DNA Isolation Kit

**Description:**
DENAzist Genomic DNA isolation kit is designed for quick and easy extraction of genomic DNA from plant tissues. Using this kit it would be possible to extract genomic DNA with the highest quality. The extracted DNA could be used in a multitude of downstream procedures including PCR, genotyping, sequencing, and different hybridization techniques including Southern blotting.

<table>
<thead>
<tr>
<th>Components</th>
<th>Applications</th>
<th>Storage</th>
<th>Shipping</th>
<th>Expiry date</th>
</tr>
</thead>
<tbody>
<tr>
<td>DG1 buffer</td>
<td>25 25 ml</td>
<td>50 ml RT</td>
<td>Shipped at ambient temperature.</td>
<td>The kit is fully active until the expiry date indicated on the box, and when all components are stored under the recommended conditions.</td>
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<tr>
<td>DG2 buffer</td>
<td>9 ml 9 ml</td>
<td>18 ml RT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DG3 buffer</td>
<td>9 ml 9 ml</td>
<td>18 ml RT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DG4 buffer</td>
<td>3 ml 6 ml</td>
<td>RT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spin columns</td>
<td>25 25</td>
<td>50 RT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Important instructions before first use**
For 25 application kit, add 4 ml isopropanol to DG2 and 12 ml ethanol (96%-100%) to DG3 containers. For 50 application kit, add 8 ml isopropanol to DG2 and 24 ml ethanol (96%-100%) to DG3 containers. Label the container to record that ethanol has been added before first use.

**PROTOCOL for isolation of genomic DNA**
- Before first use, make sure that isopropanol and ethanol have been added to the containers labeled DG2 and DG3, respectively:
  1. Grind 20 mg solid tissue sample in liquid nitrogen in a mortar and pestle. Add 10 mg ground tissue to a homogenizing vessel.
  2. Add 0.5 ml of DG1 buffer to this vessel. Add 5 microliter from 2-mercaptoethanol and mix. By vortexing and pipetting, immediately resuspend the tissue lysate in the solution.
  3. Homogenize the tissue/cell lysate by passing it through a 20-gauge needle fitted to a sterile syringe for at least 5 times.
  4. Centrifuge the tube at 10,000 rpm for 2 minutes. Transfer the supernatant into a new spin column inserted into a collecting tube (available in the kit).
  5. Centrifuge the spin column at 10,000 rpm for 1 min. Discard the flow-through accumulated in the collection tube. Remount the spin column onto the collection tube.
  6. Add a mix of 500 μl DG1 and 5 μl 2-mercaptoethanol to the spin column. Centrifuge at 10,000 rpm for 1 minute at room temperature. Discard the flowthrough.
  7. Add 500 μl from DG2 solution into the spin column. Centrifuge the spin column at 10,000 rpm for 1 min. Discard the flow-through.
  8. Add 700 μl from DG3 solution into the spin column. Centrifuge the spin column at 10,000 rpm for 1 min. Discard the flow-through.
  9. Without adding any solutions, spin the tube one more time at top speed (13,000 rpm) for 3 min.
  10. Separate the spin column from its collecting tube and place it into a new 1.5 ml microfuge (Eppendorf) tube.
  11. Add 40-100 μl from DG4 solution onto the center of spin column. Leave the spin column mounted on the microfuge tube for 5 min at room temperature. Centrifuge the spin column mounted on microfuge tube at 10,000 rpm for 2 min.
  12. Return the eluted solution from the previous step back on to the center of spin column. Leave the spin column mounted on the microfuge tube for 2 min at room temperature. Centrifuge the spin column mounted on microfuge tube at 13,000 rpm for 2 min.
  13. The eluted solution at the bottom of microfuge tube contains pure genomic DNA. Until further downstream processes store the sample in -20°C freezer.

**Notes:** Research Use Only
This product insert declares that this product has been analyzed and passed the quality control tests at the time of manufacture.