EURx® MOLECULAR BIOLOGY PRODUCTS

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Universal kit for DNA isolation with GeDI reagent.

Complete kit containing spin columns and ready-to-use GeDI reagent for the isolation of total DNA from diverse samples.

kat. nr. E3765

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Spis treści

The method is based on the precipitation of nucleic acids from a cell lysate with ethanol. The sample is homogenized and lysed in a specially composed **GeDI** solution (which does not contain organic solvents such as phenol) and after addition of ethanol DNA is selectively precipitated from the solution. The pellet solubilized in a special **ResSol** buffer can be applied on a minicolumn with silica membranes. This allows to increase purity of the isolated DNA and obtain high quality material. The precipitate can also be dissolved in 8 mM NaOH and, after neutralization, directly used for standard molecular and biotechnological applications such as: PCR, molecular cloning, RFLP, Southern blotting, etc.
Universal DNA isolation reagent (**GeDI**) is available separately *(E3760)* or as part of a kit *(E3765)* which contain silica columns, **ResSol** solution and reagents for additional sample purification.

Equipment and reagents to be supplied by the researcher.

1. Ethyl alcohol [95 100% v/v], ethyl alcohol [75% v/v], sterile DNase free water.
2. 8 mM NaOH (fresh prepared), 1 M HEPES.
3. For blood protocol – Lyse RBC buffer (Cat. no. E0326) for erythrocytes lysis. When blood volume exceeds 400 µl – appropriate size plastic tubes for erythrocytes lysis and centrifugation after lysis.
4. For tissue and plant protocol – equipment for sample disruption and homogenization, depending on the method chosen: mortar, pestle and liquid nitrogen or handheld rotor-stator homogenizer.
5. Microcentrifuge, vortex, disposable gloves, sterile pipet tips, sterile 1.5–2 ml tubes, a heating block suitable for sample incubation at 50–70°C.

Optional:

1. For disrupting small portions of plant and animal tissues **Tissue Grinding Tool** *(E0359)*.
2. For gram-positive bacteria, yeasts or tissues pre-ground with **Tissue Grinding Tool** *(E0359)*, tubes containing glass spheres for homogenization samples **BeadTubeDry** *(E0358)* and antifoaming reagent for EURx lysis buffers – **AFR01** *(E0328)*.

Introductory Notes

NOTE 1 · Application. GeDI reagent is a monophasic solution of chaotropic salts and other components (which does not contain organic solvents such as phenol) design to facilitate the efficient lysis and isolation of nucleic acids from cell and tissue samples of human, animal, plant, yeast or bacterial origin.

NOTE 2 · Maximum Sample Amount. 1.0 ml of **GeDI** reagent is sufficient to isolate DNA from a maximum of 50 mg animal tissue or 50-200 mg plant tissue or approximately 1 x 10^7 animal, yeast or bacterial cells. The sample volume should not exceed 10% of the volume of **GeDI** reagent used for homogenization. When isolating nucleic acids from human leukocytes, starting volume of blood should not exceed 1 ml per 1 ml **GeDI** reagent used for leukocytes lysis.

NOTE 3 · Homogenization and Lysis. Efficient disruption and homogenization of the starting material is required for most of samples. It can be carried out directly in **GeDI** reagent or frozen with liquid nitrogen samples should be grounded in a mortar with a pestle. For samples that are easily lysed (bacterial cells, cell cultures), it is enough to suspend the material in **GeDI** solution.

NOTE 4 · Sample Storage. After cells or tissues have been homogenized or lysed in **GeDI** reagent, samples can be stored at -20°C or -80°C for a few months.

NOTE 5 · Kit Compounds Storage. GeDI reagent and other buffers are stable at temperature 2–25°C. For long term storage, store all components of the kit at 2–8°C.

NOTE 6 · Additional Remarks. The resuspended DNA may contain partially degraded RNA. In applications requiring high purity DNA, RNase A digestion should be performed or steps described in the On column DNA purification section.

Approximate efficiency of total DNA isolation depending on the type of tissues or cells.

Starting Material	Quantity	Yield of Genomic DNA
E. coli Cells	10^9 cells	30 – 40 µg
Human Cells	10^6 cells	4 – 7 µg
Mouse Cells	10^6 cells	4 – 7 µg
Whole Blood	1 ml	20 – 40 µg
Plant Leaf	0.5 g	10 – 100 µg
Placenta	10 mg	20 – 30 µg
Skeletal Muscle	10 mg	15 – 25 µg
Brain	10 mg	15 – 30 µg
Kidney	10 mg	30 – 40 µg
Mouse Tail	10 mg	4 – 30 µg
Lung	10 mg	30 – 50 µg
Heart	10 mg	15 – 30 µg
Liver	10 mg	30 – 40 µg

DNA isolation

Part I Disruption and sample lysis

1. **Animal tissue:**
   Homogenize a tissue fragment in **GeDI** reagent. Keep the ratio: maximum 50 mg of a tissue per 1 ml of a reagent.
   Tissue can be frozen with liquid nitrogen and then thoroughly ground in a chilled mortar with a pestle. Add the powder to **GeDI** reagent and mix well.
   In case of additional purification on minicolumns or in case of use efficient mechanical homogenizers, up to 10 times less tissue should be used.
   For the homogenization of small portions of tissues we recommend **Tissue Grinding Tool** *(E0359)* – see page 10.

2. **Plant tissue:**
   Homogenize a plant tissue fragment in **GeDI** reagent. Keep the ratio: maximum 50 – 200 mg of a tissue per 1 ml of a reagent.
   Tissue can be frozen with liquid nitrogen and then thoroughly ground in a chilled mortar with a pestle. Add the powder to **GeDI** reagent and mix well.
   In case of additional purification on minicolumns or in case of use efficient mechanical homogenizers, up to 10 times less tissue should be used.
   For the homogenization of small portions of tissues we recommend **Tissue Grinding Tool** *(E0359)* – see page 10.

3. **Cell grown in suspension:**
   Pellet cells by centrifugation and remove media. Lyse cells with 1 ml of **GeDI** reagent per maximum 1 x 10^7 cells and pass the lysate several times through a pipette tip.

4. **Cell grown on monolayer:**
   Remove growth media. Lyse cells directly in a culture dish or flask by adding 1 ml of **GeDI** reagent per 10 cm^2 growth area. Pipette the cell lysate several times to ensure sufficient cell disruption.

5. **Blood (leukocytes):**
   GeDI reagent can be used for DNA isolation from leukocytes. The maximum amount of human blood is 1 ml per 1 ml **GeDI** reagent.
   Add 4 volumes of buffer **Lyse RBC** *(E0326)* to a whole blood. Mix by inverting the tube. Keep at 4°C for 10 minutes to lyse erythrocytes. Mix twice by inverting the tube. Centrifuge at 1000 x g for 5 minutes at 4°C, and carefully decant the supernatant.
   Add **GeDI** reagent to the leukocytes pellet. Mix thoroughly by pipetting for homogenization.

6. **Bacterial cells:**

Gram negative: Pellet bacteria from a suitable culture volume by centrifugation and remove media. Lyse cells with 1 ml of **GeDI** reagent per maximum 1 x 10^8 cells and pass the lysate several times through a pipette tip.

Gram positive: Freeze the bacterial pellet with liquid nitrogen and then ground to a powder. Suspend the appropriate amount of powder in 1 ml of **GeDI** reagent. Use 1 ml of **GeDI** reagent for maximum 1 x 10^8 cells and pass the lysate several times through a pipette tip.

Homogenization with **BeadTubeDry**: Add the foam reducing reagent for the EURx lysis solutions (**AFR01**) to **GeDI** reagent – 50 µl **AFR01** per 10 ml **GeDI**. Pellet bacteria from a suitable culture volume by centrifugation and remove media. Lyse cells with 1 ml of **GeDI** reagent. Transfer the mixture to a **BeadTubeDry** with glass beads. Place **BeadTubeDry** in the vortex and shake for 10 min at maximum speed.

- *For tube shaking, specialized bead beater/cell disrupter instruments (e.g. FastPrep, Precel-lys, Disruptor Genie, etc.) can be used to achieve greater efficiency in DNA isolation. But, for preventing excessive DNA fragmentation, it is required to optimize the shaking time (generally, a time reduction, as compared to the time specified above for vortexing, depending on the specific type of cell disruptor in use).*

After homogenization step, in case of high foaming, the sample should be centrifuged at 8 000 x g for 30 sec. After centrifugation, transfer the maximum possible volume of the supernatant to a new tube. Note the volume obtained.

7. **Yeast:**

Use the protocol for Gram positive bacteria.

Part II DNA Precipitation

1. Incubate homogenized sample at 70°C for 15 min.
2. Shake the sample by hand or using the vortex for 5 sec.
3. Centrifuge the sample at room temperature for 2 min at 10 000 x g.

   - *The cell debris and non-homogenized fragments of tissues will be deposited. For the next isolation step, take a clear supernatant.*

4. Transfer the supernatant to a new tube and add 1 ml of ethyl alcohol (95–100% v/v). In the case of homogenization with **BeadTubeDry**, add a volume of ethanol equal to the volume of the supernatant collected. Leave at room temperature for 3–5 min.

   - *The DNA should become visible as a cloudy precipitate. Precipitated DNA will not be visible if concentration is low (<10 µg) or the DNA is fragmented.*

   - *In some cases it is possible to spool the precipitated DNA by swirling onto a pipette tip and transfer the spooled DNA to a clean tube. Then rinse DNA pellet with 75% ethanol, remove ethanol, dry the precipitate and suspend the DNA in the appropriate buffer. If spooling DNA is not possible, continue with following protocol steps.*

5. Centrifuge sample at room temperature for 2 min at 10 000 x g. Discard the supernatant, ensuring that all liquid is completely removed.
6. Depending on your needs, go to the next part of the protocol (On column DNA purification), or follow the points below.
7. Wash the pellet once with 1 ml 75% ethanol and vortex the sample briefly. Centrifuge sample at room temperature for 2 min at 10 000 x g. Discard the supernatant, ensuring that all liquid is completely removed.
8. Air-dry the pellet and dissolve in 8 mM NaOH. Use the right volume depending on your needs. Incubate samples for 5 min at room temperature. Mix well. If necessary, briefly heat the sample at 55°C and mix thoroughly again. To remove insoluble material centrifuge sample at room temperature for 2 min at 10 000 x g. Transfer the cleared supernatant to a new tube and neutralize with a suitable amount of 1 M HEPES.

pH adjustment of DNA samples dissolved in 8 mM NaOH.
For 1 ml of 8 mM NaOH, use the following amounts of 1M HEPES (free acid):

1 M HEPES [µl]	Final pH
51.5	7.0
36.5	7.2
22.5	7.5
17.5	7.7
12.5	8.0
10.5	8.2
9.0	8.5

- *Weak alkaline solutions (as 8 mM NaOH) are neutralized by CO_2 from the air. It is recommended to prepare 8 mM NaOH solution fresh, once a month.*

- *The resuspended DNA may contain partially degraded RNA. In applications requiring high purity DNA, RNase A digestion should be performed or steps described in the On column DNA purification section.*

Part III On column DNA purification

1. Apply 30 µl of activation **Buffer BG** onto the **DNA binding spin-column** (do not spin) and keep it at room temperature till transfering lysate to the spin-column.

   - *Addition of Buffer BG onto the center of the resin enables complete wetting of membranes and maximal binding of DNA.*

   - *The membrane activation should be done before starting isolation procedure.*

2. Suspend the precipitate obtained at step 5 part II of the protocol (DNA Precipitation) in 350 µl of **ResSol** buffer.

3. Incubate samples for 5 min at room temperature. Mix well. If necessary, briefly heat the sample at 55°C and mix thoroughly again. To remove insoluble material centrifuge sample at room temperature for 2 min at 10 000 x g.

4. Carefully transfer the clear supernatant to a **DNA binding spin-column**.

   - *Pay attention not to stir up any pellet that may have formed during the spin down cycle.*

5. Centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back the spin-column into the receiver tube.

6. Add 550 µl of **Wash BGX** buffer and centrifuge at 11 000 x g for 1 min. Remove the spin-column, discard the flow-through and place back the spin column into the receiver tube.

7. Add 300 µl of **Wash BGX** buffer and centrifuge at 11 000 x g for 2 min.

   - *Be careful not to contaminate the sample while removing the spin-column from the receiver tube. Check, whether the membrane of the spin column is completely dry. If not, pour off any remaining supernatant and place back spin-column into the receiver tube. Spin down for one additional min.*

8. Place spin-column into a new receiver tube (1.5–2 ml) and add 60–150 µl of **Elution** buffer to elute bound DNA. Incubate for 2 min at room temperature.

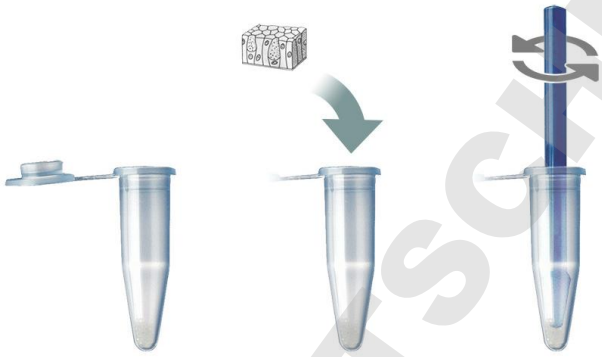
   - *Addition of the elution buffer directly onto the center of the resin improves DNA yield. To avoid transferring traces of DNA between the spin-columns do not touch the spin-column walls with the micro-pipette.*

   - *In order to improve the efficiency of the elution genomic DNA from membrane, elution buffer can be heated to a temperature of 80°C.*

9. Centrifuge for 2 min at 11 000 x g. Remove spin-column, cap the receiver tube. Genomic DNA is ready for analysis/manipulations. It can be stored either at 2–8°C (preferred) or at -20°C (avoid multiple freezing and defrosting of DNA).

Homogenization of small portions of tissues using TGT

Tissue Grinding Tool (TGT) *(E0359)* is a convenient tool for disrupting small portions of plant and animal tissues, bacterial, yeast or cell cultures pellets in quantities corresponding to one extraction experiment. The set consists of a 1.5 ml Eppendorf tube containing a small amount of grinding beads and a pestle with a specially adapted shape. The sample can be homogenized in a small amount of lysis buffer or without any additives. In most cases, a better homogenization effect can be obtained by grinding the material in a small volume of lysis solution (50–150 µl). **TGT** can also be used to disperse centrifugal pellets, precipitates, and other soft solids.



1. Add 50–150 µl of **GeDI** reagent to the tube with selected grinding beads. Then add an appropriate portion of plant or animal tissue. Crush the sample by rotating the pestle with your fingers. The grinding time depends on the type of sample and varies from 30 sec to a maximum of 2 min.
2. Remove the pestle, fill the volume of **GeDI** reagent to 1 ml. Vortex for a few seconds or mix thoroughly by inverting the tube.

   - *For better sample fragmentation, homogenization ca be continued using BeadTubeDry. To do this, transfer the pre-ground sample to BeadTubeDry and continue the protocol as in the case described for gram positive bacteria "Homogenization with BeadTubeDry".*

3. Continue with step 1 Part II of the Protocol (DNA Precipitation).

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