

## GeneMATRIX DNA/RNA Extracol Kit

Phenol-based reagent for the isolation of total RNA and DNA from cells and tissues in a set with minicolumns.

○ **Cat. no. E3750**

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# Introductory Notes

**NOTE 1 • Application.** DNA/RNA Extracol is a reagent for the isolation of total RNA and DNA from cell and tissue samples of human, animal, plant, yeast or bacterial origin. DNA/RNA Extracol is a monophasic solution of phenol, chaotropic salts and other components design to facilitate the isolation of nucleic acids.

**NOTE 2 • Maximum Sample Volume.** 1.0 ml of DNA/RNA Extracol is sufficient to isolate DNA and RNA from a maximum of 100 mg tissue or is sufficient to lyse  $5-10 \times 10^6$  animal, plant or yeast cells or  $1 \times 10^7$  bacterial cells. The sample volume should not exceed 10% of the volume of DNA/RNA Extracol used for homogenization. When isolating nucleic acids from human leukocytes, starting volume of blood should not exceed 1.5 ml per 1 ml DNA/RNA Extracol used for leukocytes lysis. The maximum column binding capacity for DNA is 25  $\mu\text{g}$ . Loading more than 25  $\mu\text{g}$  DNA may lead to DNA contamination of the RNA eluate. The maximum column binding capacity for RNA is 125  $\mu\text{g}$ . The maximum volume of the column reservoir is 650  $\mu\text{l}$ .

**NOTE 3 • Homogenization and lysis.** Efficient disruption and homogenization of the starting material is requirement for most kind of samples. It can be carried out directly in DNA/RNA Extracol or in RL buffer (E0310). When using RL buffer, volume of homogenized tissue should not exceed 10% of the volume of DNA/RNA Extracol used for DNA/RNA isolation.

**NOTE 4 • Sample Storage.** After the cells or tissues have been homogenized or lysed in DNA/RNA Extracol, samples can be sotred at  $-80^\circ\text{C}$  for at least one month.

**NOTE 5 • Kit Compounds Storage.** Keep all solutions tightly closed to avoid evaporation, resulting in components concentration changes. DNA/RNA Extracol solution and other buffers are stable at temperature  $2-25^\circ\text{C}$ . For long term storage, store all components of the kit at  $2-8^\circ\text{C}$ .

**NOTE 6 • Caution.** DNA/RNA Extracol contains phenol (toxic and corrosive) and guanidine isothiocyanate (irritant) and may be a health hazard if not handled properly. Always work with DNA/RNA Extracol in a fume hold and always wear a lab coat, gloves and safety glasses.

The method is based on the extraction of nucleic acids aqueous solutions using organic solvents. After homogenizing the sample with DNA/RNA Extracol, chloroform (or 1-bromo-3-chloropropane) is added, and the homogenate is allowed to separate into a clear upper aqueous layer, an interphase, and a lower organic layer. Separation of nucleic acids between the phases is pH dependent. At pH higher than 6 RNA and DNA remains in the aqueous phase. The highly effective RNase inhibitory property of DNA/RNA Extracol protects the integrity of the DNA/RNA during lysis and results in the isolation of high-quality material. Nucleic acids can be precipitated from the aqueous layer with isopropanol or separeted into DNA and RNA fraction using minicolumns and wash buffers.

### Equipment and reagents to be supplied by user:

1. Chloroform or 1-bromo-3-chloropropane, isopropanol, ethanol 75%, ethanol 96–100%.
2. Optional: RL buffer (E0310) for sample homogenization.
3. Optional: Lyse RBC buffer (E0326) for erythrocytes lysis.
4. Refrigerated laboratory centrifuge or microcentrifuge, disposable gloves, sterile RNase-free pipet tips, sterile RNase-free 1.5–2 ml tubes, vorteks, equipment for sample disruption and homogenization.

## DNA/RNA isolation

### Part I Disruption and sample lysis

#### 1. Tissue:

Homogenize tissue samples in 1 ml of **DNA/RNA Extracol** per 10–100 mg of tissue.

The tissue can also be homogenized in **RL** buffer (E0310). When using **RL** buffer for homogenization, in the next step, suspension of homogenized tissue must be added to **DNA/RNA Extracol** solution. Volume of homogenized tissue should not exceed 10% of the volume of **DNA/RNA Extracol** used for DNA/RNA isolation. For samples of fat tissue, a layer of fat may accumulate at the top, which should be removed. Centrifuge sample at 12 000 x g for 10 min at 4°C. Remove and discard the fatty layer.

#### 2. Plant tissue:

Homogenize plant tissue samples in 1 ml of **DNA/RNA Extracol**.

The tissue can also be homogenized in **RL** buffer (E0310). When using **RL** buffer for homogenization, in the next step, suspension of homogenized tissue must be added to **DNA/RNA Extracol** solution. Volume of homogenized tissue should not exceed 10% of the volume of **DNA/RNA Extracol** used for DNA/RNA isolation. For samples with high content polysaccharides or extracellular material, an additional centrifugation step is required to remove insoluble material from the sample. Centrifuge sample at 12 000 x g for 10 min at 4°C. Transfer the cleared supernatant to a new tube.

#### 3. Cell grown in suspension:

Pellet cells by centrifugation and remove media. Lyse cells with 1 ml of **DNA/RNA Extracol** per 5–10 x 10<sup>6</sup> 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 **DNA/RNA Extracol** per 10 cm<sup>2</sup> growth area. Pipette the cell lysate several times to ensure sufficient cell disruption.

○ *DNA/RNA Extracol is not compatible with plastic culture plates.*

## 5. Blood (leukocytes):

**DNA/RNA Extracol** can be used for DNA/RNA isolation from leukocytes. The maximum amount of human blood is 1.5 ml per 1 ml **DNA/RNA Extracol**. If the main purpose is the isolation of RNA, do not use frozen blood.

Add 4 volumes of buffer **Lyse RBC** (E0326) to a fresh blood. Mix by inverting the tube. Keep at 4°C for 10 min to lyse erythrocytes. Mix twice by inverting the tube. Centrifuge at 400 x g for 10 min at 4°C, and carefully decant the supernatant.

Add **DNA/RNA Extracol** to the leukocytes pellet. Mix thoroughly by pipetting for homogenization.

## Part II Phase Separation

1. Incubate samples for 5 min at room temperature.
2. Add 0.2 ml of chloroform (or 0,1 ml 1-bromo-3-chloropropane) per 1 ml of **DNA/RNA Extracol** used for homogenization.
3. Cover the sample tightly, shake vigorously for 15 sec.
4. Incubate samples for 2–5 min at room temperature.
5. Centrifuge sample at 12 000 x g for 15 min at 4°C.
  - Centrifugation separates the mixture into 3 phases: orange organic phase (containing protein), an interphase and a colorless upper aqueous phase (containing DNA and RNA). The upper aqueous phase is ~50% of the total volume.
6. Remove the aqueous phase very carefully, without disturbing the interphase. For isolation of both, DNA and total RNA, continue with part III of the protocol DNA/RNA Precipitation. For separation of DNA from RNA continue with the steps described in part IV of the protocol DNA Purification.

## Part III DNA/RNA Precipitation

1. Add 0.5 ml of 100% isopropanol to the aqueous phase, per 1 ml of **DNA/RNA Extracol** used for homogenization.
2. Incubate samples for 10 min at room temperature.
3. Centrifuge sample at 12 000 x g for 10 min at 4°C.
4. Remove the supernatant. Wash the pellet once with 1 ml 75% ethanol per 1 ml of **DNA/RNA Extracol** used in the initial homogenization. Vortex the samples briefly.
5. Centrifuge sample at 10 000 x g for 5 min at 4°C.
6. Remove the supernatant. Vacuum or air-dry the pellet and dissolve in **RNase-free water**.
  - Sample contains DNA, and RNA.

## Part IV DNA Purification

1. Apply 30  $\mu$ l of activation **Buffer A** onto the **DNA binding spin-column** (do not spin) and keep it at room temperature till transferring aqueous phase to the spin-column (for best results at least 10 min).
  - *Addition of Buffer A 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. The aqueous phase from the last step of part II of the protocol (**Phase Separation**) transfer to the **DNA binding spin-column** placed in a 2 ml receiver tube. Centrifuge at 12 000 x g for 1 min. **Use the flow-through for RNA purification.**
  - *To receive RNA only, keep the flow-through and continue with part V of the protocol (**RNA Purification**).*
3. Add 600  $\mu$ l of **Wash RBW** buffer to the DNA binding spin column and centrifuge at 12 000 x g for 1 min.
4. Remove the spin-column, pour off supernatant and place back into the receiver tube.
5. Add 300  $\mu$ l of **Wash RBW** buffer to the spin column and centrifuge at 12 000 x g for 2 min.
  - *Be careful not to contaminate the sample while removing spin-column from receiver tube. Check whether column is completely dry. If not, pour off supernatant and place back spin-column into the receiver tube and spin down for additional 1 min.*
6. Place spin-column into new receiver tube (1.5–2 ml) and add 50–100  $\mu$ l of **Elution** buffer directly onto the membrane to elute bound DNA. Incubate spin-column/receiver tube assembly for 2 min at room temperature.
  - *In order to improve the efficiency of the elution genomic DNA from membrane, Elution buffer can be heated to a temperature of 80°C.*
7. Centrifuge for 1 min at 12 000 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).

## Part V RNA Purification

1. To the flow-through from the step 2 of part IV of the protocol (**DNA Purification**) add the same volume of ethanol (96–100% [v/v]). Mix thoroughly. Do not centrifuge.
  - For example, if 400  $\mu$ l flow-through was collected in step 1 in Part IV of the protocol, add 400  $\mu$ l ethanol.
2. Apply up to 600  $\mu$ l of the mixture to the **RNA binding spin-column** and centrifuge at 12 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.
3. Transfer the remaining mixture to the same **RNA binding spin-column** and centrifuge at 12 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.
4. Add 600  $\mu$ l of **Wash RBW** buffer to the RNA binding spin column and centrifuge at 12 000 x g for 1 min.
5. Remove the spin-column, pour off supernatant and place back into the receiver tube.
6. Add 300  $\mu$ l of **Wash RBW** buffer to the spin column and centrifuge at 12 000 x g for 2 min.
  - Be careful not to contaminate the sample while removing spin-column from receiver tube. Check whether column is completely dry. If not, pour off supernatant and place back spin-column into the receiver tube and spin down for additional 1 min.
7. Place spin-column into new receiver tube (1.5–2 ml) and add 50–100  $\mu$ l of **RNase-free water** directly onto the membrane to elute bound RNA. Incubate spin-column/receiver tube assembly for 2 min at room temperature.
8. Centrifuge for 1 min at 12 000 x g. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. Store the samples at -20°C or below.

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