EURx® MOLECULAR BIOLOGY PRODUCTS

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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 x 10^6 animal, plant or yeast cells or 1 x 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 µg. Loading more than 25 µg DNA may lead to DNA contamination of the RNA eluate. The maximum column binding capacity for RNA is 125 µg The maximum volume of the column reservoir is 650 µl.

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°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°C. For long term storage, store all components of the kit at 2–8°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 10^6 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^2 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 µl of activation **Buffer A** onto the **DNA binding spin-column** (do not spin) and keep it at room temperature till transfering 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 µl 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 µl 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 µl 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 µl flow-through was colected in step 1 in Part IV of the protocol, add 400 µl ethanol.*

2. Apply up to 600 µl 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 µl 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 µl 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 µl 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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