EURx® MOLECULAR BIOLOGY PRODUCTS

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Version 3.1
October 2019

GeneMATRIX DNA/RNA Extracol Kit

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

Cat. no. E3750

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Table of Contents

Content	25 preps E3750-01	100 preps E3750-02	Storage/Stability
DNA/RNA Extracol*	30 ml	120 ml	2-25°C
Buffer A	0.9 ml	3.6 ml	15-25°C
Wash RBW	54 ml	216 ml	15-25°C
PLB	4.5 ml	18 ml	15-25°C
Bromophenol Blue 0.5%	0.1 ml	0.4 ml	15-25°C
RNase-free water	3.6 ml	15 ml	15-25°C
Elution	3.6 ml	15 ml	15-25°C
DNA Binding Columns	25	2 x 50	2-25°C
RNA Binding Columns	25	2 x 50	2-25°C
Protocol	1	1	

* 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. It can be stored both at room temperature and at 2-8°C (fridge).

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

- Chloroform or 1-bromo-3-chloropropane, isopropanol, ethanol 75%, ethanol 96-100%.
- Optional: RL buffer (E0310) for sample homogenization.
- Optional: Lyse RBC buffer (E0326) for erythrocytes lysis.
- 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.
- For protein precipitation from tissue, cell culture, blood (leukocytes): GE solution – 0.3 M guanidine hydrochloride in 95% ethanol, either β-mercaptoethanol (14.3 M, β-ME) or [1 M] Dithiothreitol (DTT).
- For protein precipitation from plant tissue: either β-mercaptoethanol (14.3 M, β-ME) or [1 M] Dithiothreitol (DTT).

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**.
   Optionally, keep the residue (interphase and the bottom phenolic layer) to isolate the proteins. For protein purification, go to **Part VI Protein Precipitation**.

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.

Part VI Protein precipitation

Tissue, cell culture, blood (leukocytes)

1. To the phenol phase (step 6 in part II of the protocol), add 1.5 volumes of isopropanol. Mix thoroughly. Incubate for 10-30 min at room temperature.
2. Centrifuge at 12 000 x g for 10 min at 4°C, and carefully decant the supernatant.
3. To the protein pellet add 1 ml of GE solution, per 1 ml of DNA/RNA Extracol used for homogenization. Mix thoroughly. Incubate for 10-20 min at room temperature.

   - *Prepare a wash solution GE consisting of 0.3 M guanidine hydrochloride in 95% ethyl alcohol.*

4. Centrifuge at 7 500 x g for 5 min at 4°C, and carefully decant the supernatant.
5. Repeat steps 3 and 4.
6. Wash the pellet once with 2 ml of ethanol (96-100%). Vortex the samples briefly.
7. Centrifuge at 7 500 x g for 5 min at 4°C, and carefully decant the supernatant.
8. Dry the protein pellet for 5-15 min at room temperature.
9. Dissolve the protein pellet in 80-150 µl protein loading buffer **PLB** (Note 2).

   - *Buffer PLB is a sample buffer for use in SDS-PAGE analysis. If the proteins will not be analyzed by SDS-PAGE, use a buffer compatible with the intended application. As a result of the method of isolation the precipitated protein is highly denatured and shows reduced solubility in water. Dissolution the precipitate is possible in PLB buffer or other solution containing a high concentration of detergent (eg 1.5-5% SDS). Therefore, Bradford and Lowry assays are not applicable for quantifying protein yield. For protein quantitation, use the Bicinchoninic Acid Assay (BCA).*

   - *For SDS-PAGE analysis, add 10 µl Bromophenol Blue and either 25 µl β-mercaptoethanol (β-ME) or 25 µl [1 M] dithiothreitol per 1 ml buffer PLB before use. After addition of β-ME store buffer PLB at 2-8°C. DTT-supplemented buffer PLB must not be stored, always prepare fresh aliquots.*

   - *In case of PLB buffer ingredients precipitation warm up until clarified.*

10. Incubate for 5-10 min at 60°C to dissolve and denature sample.
11. If some insoluble material is still visible, centrifuge at maximum speed for 1 min. The supernatant is ready to use in downstream applications such as SDS-PAGE and others.

    - *Sample can be stored at 2-8°C for short period or at -20°C for several months.*

Plant tissue

1. To the phenol phase (step 6 in part II of the protocol), add 2 volumes of ethanol (96-100%). Mix thoroughly. Incubate for 30 min at 4°C.
2. Centrifuge at 12 000 x g for 10 min at 4°C, and carefully decant the supernatant.
3. Wash the pellet with 2 ml of 75% ethanol per 1 ml of **DNA/RNA Extracol** used in the initial homogenization. Vortex the samples briefly.
4. Centrifuge at 7 500 x g for 5 min at 4°C, and carefully decant the supernatant.
5. Repeat steps 3 and 4.
6. Dry the protein pellet for 5-15 min at room temperature.
7. Dissolve the protein pellet in 80-150 µl protein loading buffer **PLB** (Note 2).

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   - *Sample can be stored at 2-8°C for short period or at -20°C for several months.*

Safety Information

DNA/RNA Extracol




Danger

H301+**H311**+**H331** Toxic if swallowed, in contact with skin or if inhaled.
H314 Causes severe skin burns and eye damage.
H341 Suspected of causing genetic defects.
H373 May cause damage to organs through prolonged or repeated exposure.
H411 Toxic to aquatic life with long lasting effects.
P273 Avoid release to the environment.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P301+**P310** If swallowed: immediately call a poison center/doctor.
P302+**P352** If on skin: wash with plenty of water.
P304+**P340** If inhaled: remove person to fresh air and keep comfortable for breathing.
P305+**P351**+**P338** If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P405 Store locked up.
EUH032 Contact with acids liberates very toxic gas.

Buffer A

Danger

H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P301+**P330**+**P331** If swallowed: Rinse mouth. Do not induce vomiting.
P303+**P361**+**P353** If on skin (or hair): take off immediately all contaminated clothing. Rinse skin with water [or shower].
P305+**P351**+**P338** If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a poison center/doctor.
P405 Store locked up.

Wash RBW


Danger

H225 Highly flammable liquid and vapour.
H319 Causes serious eye irritation.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P305+**P351**+**P338** If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P403+**P235** Store in a well-ventilated place. Keep cool.
P337+**P313** If eye irritation persists: Get medical advice/ attention.

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SELECTION OF THE KITS DEPENDING ON THE TYPE OF ISOLATED MATERIAL

			ISOLATION OF DNA																			
			E3600	E3585	E3540	E3580	E3510	E3545	E3560	E3555	E3525	E3520	E3595	E3535	E3500	E3565	E3515	E3570	E3575	E3530	E3550	E3551
			MICELLULA DNA[2]	GRAM PLUS & YEAST GENOMIC DNA	AGAROSE – OUT DNA	BACTERIAL & YEAST GENOMIC DNA	BIO – TRACE DNA	BASIC DNA	BONE DNA	CELL CULTURE DNA	FOOD EXTRACT DNA	PCR / DNA CLEAN-UP	PLANT & FUNGI DNA	AGROBACTERIUM PLASMID DNA	PLASMID MINIPREP DNA	QUICK BLOOD DNA	SHORT DNA CLEAN-UP	SOIL DNA	STOOL DNA	SWAB-EXTRACT DNA	TISSUE DNA	TISSUE & BACTERIAL DNA
			AVAILABLE NUMBER OF ISOLATION (PREPS)																			
			50 150	25 100	50 150	50 150	25 100	50 150	25 50	50 150	25 100	50 150	50 150	50 150	50 150	50 150	25 100	50 100	50 100	25 100	50 150	50 150
DNA	GENOMIC	BACTERIA		●		●																●
		YEAST		●		●																
		CELL CULTURE								●											●	●
		PLANT											●									
		FUNGI											●									
		PLANT RICH IN POLYSACCHARIDES [1]											●									
		BLOOD														●						
		SOIL																●				
		STOOL																	●			
		SWAB																		●		
		ANIMAL TISSUES																			●	●
		FFPE TISSUE SECTIONS																			●	●
		RODENT TAILS																			●	●
		HAIR																			●	●
		INSECTS																			●	●
		URINE																			●	●
		BONE							●													
		BIOLOGICAL TRACES					●															
		FOOD									●											
	PLASMID	BACTERIA						●						●	●							
		YEAST				●																
	ISOLATION FROM AGAROSE GELS				●			●														
	PURIFICATION OF PCR PRODUCTS / DNA AFTER ENZYMATIC REACTIONS		●					●				●					●					

All kits contain buffers WASH in ready to use form

1. Additionally required lyse CT buffer (E0324)
2. Kit for creation of emulsions and subsequent DNA purification.

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