EURx® MOLECULAR BIOLOGY PRODUCTS

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RNA Extracol

Phenol-based reagent for the isolation of total RNA from cells and tissues.

Cat. no. E3700

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Introductory Notes

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

NOTE 2 · Maximum Sample Volume. 1.0 ml of RNA Extracol is sufficient to isolate 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 RNA Extracol used for homogenization. When isolating RNA from human leukocytes, starting volume of blood should not exceed 1.5 ml per 1 ml RNA Extracol used for leukocytes lysis.

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 RNA Extracol or in RL buffer (E0310). When using RL buffer, volume of homogenized sample should not exceed 10% of the volume of RNA Extracol used for RNA isolation.

NOTE 4 · Sample Storage. After the cells or tissues have been homogenized or lysed in RNA Extracol, samples can be sotred at -80°C for at least one month.

NOTE 5 · Kit Compounds Storage. RNA Extracol solution is stable at temperature 2–25°C. For long term storage, keep solution at 2–8°C.

NOTE 6 · Caution. RNA Extracol contains phenol (toxic and corrosive) and guanidine isothiocyanate (irritant) and may be a health hazard if not handled properly. Always work with 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 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 4–6 DNA passes into the organic phase while RNA remains in the aqueous phase (containing RNA). The highly effective RNase inhibitory property of RNA Extracol protects the integrity of the RNA during lysis and results in the isolation of high-quality material. RNA is precipitated from the aqueous layer with isopropanol. The precipitated RNA is washed to remove impurities and then resuspended for use in downstream applications.

Equipment and reagents to be supplied by user:

1. Chloroform or 1-bromo-3-chloropropane, isopropanol, ethanol 75%, Rnase-free water.
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.

RNA isolation

In the case of isolation of RNA from samples stabilized in fix RNA reagent (E0280), be sure to separate sample material from fix RNA reagent before proceeding with the RNA Extracol procedure. For tissue samples, the sample can be drawn from the fix RNA reagent using sterile tweezers before placing in the appriopriate lysis buffer (RNA Extracol or RL buffer). In case of particulate, granular consistence of sample material (very small pieces/particles) or for cell cultures, bacteria cultures or leukocytes, proceed as follows: Immediately prior to isolation, add one part of RNase free water to two parts of fix RNA reagent. Centrifuge, carefully decant the supernatant and remove any remaining solution by careful pipetting. Add an appriopriate amount of RNA Extracol or RL buffer to the pelleted tissue and follow the given protocol for isolation of RNA. Detailed description how to proceed with samples stabilized in fix RNA reagent can be found in the protocol available at www.eurx.com.pl.

Part I Disruption and sample lysis

1. **Tissue:**
   Homogenize tissue samples in 1 ml of **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 **RNA Extracol** solution. Volume of homogenized tissue should not exceed 10% of the volume of **RNA Extracol** used for 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 **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 **RNA Extracol** solution. Volume of homogenized tissue should not exceed 10% of the volume of **RNA Extracol** used for 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 **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 **RNA Extracol** per 10 cm^2 growth area. Pipette the cell lysate several times to ensure sufficient cell disruption.

   - *RNA Extracol is not compatible with plastic culture plates.*

5. **Blood (leukocytes):**
   RNA Extracol can be used for RNA isolation from leukocytes. The maximum amount of human blood is 1.5 ml per 1 ml **RNA Extracol**. 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 **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 **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.

   o *Centrifugation separates the mixture into 3 phases: violet organic phase, an interphase and a colorless upper aqueous phase (containing RNA). The upper aqueous phase is ~50% of the total volume.*

6. Remove the aqueous phase very carefully, without disturbing the interphase. Place the aqueous phase into new tube and proceed to the RNA Precipitation procedure.

Part III RNA Precipitation

1. Add 0.5 ml of 100% isopropanol to the aqueous phase, per 1 ml of **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 **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**.

   o *We recommend cleanup of the redissolved RNA using Universal RNA (E3598), Universal RNA/miRNA (E3599) EURx Kits, which are based on silica-membrane technology, in order to remove any contaminating phenol. The presence of residual phenol can result in overestimation of RNA yield and inhibition of enzymatic action in downstream applications. The removal of contaminants by RNA cleanup also improves the stability of the RNA during storage. Alternatively, for RNA isolation, DNA/RNA Extracol (E3750) can be used which comprises in the procedure, purification of DNA and RNA on silica-minicolumns.*

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SELECTION OF THE KITS DEPENDING ON THE TYPE OF ISOLATED MATERIAL

				ISOLATION OF RNA					
				E3700	E3594	E3596	E3598	E3599	E3593
				RNA EXTRACOL [2]	UNIVERSAL BLOOD RNA	HUMAN BLOOD RNA	UNIVERSAL RNA	UNIVERSAL RNA /miRNA	FFPE RNA Purification Kit
				PREPS					
				25 100	25	25	25 100	25 100	25 100
RNA	TOTAL RNA LONGER THAN 200 BASES	ANIMAL TISSUE					●	●	
		PLANT TISSUE					●	●	
		BACTERIA					●		
		YEAST					●		
		CELL CULTURE					●	●	
		HUMAN BLOOD	FRESH	●	●	●	●		
			FROZEN [1]		●				
		ANIMAL BLOOD	FRESH	●	●				
			FROZEN [1]		●				
	miRNA OR TOTAL RNA	ANIMAL TISSUE		●				●	
		FFPE TISSUE SECTIONS							●
		PLANT TISSUE		●				●	
		CELL CULTURE		●				●	
		BACTERIA		●					
		YEAST		●					
		BLOOD/LEUKOCYTES		●					
	PURIFICATION OF RNA AFTER ENZYMATIC REACTIONS						●	●	
	ON-COLUMN DNase DIGESTION				●		●		

All kits contain buffers WASH in ready to use form

1. Frozen with the addition of Lyse Blood buffer (included in kit).
2. Phenol-based reagent for isolation RNA.

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