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PRODUCT INFORMATIOIN

Column-zol RNA micro isolation Kit

(Cat#704)

Kit Contents:

Components	704	Storage
Column-zol* reagent CZ additive RNA Tini Spin Column (with collection tubes)**	25 ml 0.15 ml 50 columns+100 collection tubes	18 months at room temperature For longer storage, keep all contents at 4^0C.

*contains phenol and guanidine salts. Please read **cautions** for details.

**Bulk RNA Tini Spin columns (EZC107) are sold separately for leftover solutions. For larger sample use Mini spin column (EZCR101)

Reagents and equipment supplied by the user:

- RNase-free Ethanol (100%)
- Centrifuge for micro-centrifuge tubes
- RNase-free micro-centrifuge tubes
- Manual micro-pipettes and sterile, RNase-free tips
- Equipment for sample disruption
- RNase free water
- Leak-prove, DNase/RNase free screw cap tubes (EZC165SN or EZC200SN)

Principle:

This kit combines the **Advantages** of chemical extraction method (phenol/guanidine salts) with solid-phase extraction method (immobilization glass filter embedded spin column) to provide a fast purification of high yield, high quality total RNA (including small RNAs, 17-200nt). In addition, it includes an improved RNA reagent (lysis & extraction solution with phenol/guanidine) and CZ additive to efficiently protect the RNA and remove the DNA contamination during RNA isolation. The purified RNA is **RT ready** without DNase treatment. With our new Tini RNA column (EZC107), you can now process very small samples to recover trace amount of both small and large RNA together or in separate fractions. The kit can be used for broad range of samples including cultured cells, animal tissues, plants, blood sample, body fluids, bacterial and viral origin. The recommended sample size is $<1x10^7$ cell and <100mg tissue for Mini Spin column and **$<5x10^5$ cell and <5mg tissue** for Tini spin column. The RNA Tini spin column can also be used as RNA concentrator since the elution volume is as low as 5µl. **DNA can be recovered from the pellet as well** (contact us for protocol)!

Features:

√Quick, Spin column purification of High quality RNA that is ready for RT PCR without DNase treatment.

√Bypass chloroform separation

√Avoid ethanol precipitation procedures which are time consuming and low recovery for 1) small RNA, 2) small amount RNA from small number of cells or tissues.

√Used for broad range of samples: cells, tissues, blood, body fluids, bacterial, viral origin, and plants.

√ Economic: Columns (Cat#EZCR101 and EZC107) are compatible with all other silica base RNA kits on the market and can be used as economic substitutes. It also works with any acid-guandinium-phenol based reagent such as **TRIzol, QIAzol, TriPure, and TriSure**.

Note:

Care must be taken when working with RNA. It is important to maintain an RNAse-free environment starting with RNA sample preparation and continue through purification and analysis. Use RNAse free tubes, tips, gels. Wear gloves at all times. Change gloves frequently to avoid contaminating samples with RNases. RNA is exposed to RNA-degrading enzymatic activity until the sample is frozen or disrupted using RNase-inhibiting agents. Plant and animal tissue samples should be flash frozen in liquid nitrogen immediately and stored at –70°C or processed as soon as possible.

Cautions:

Column-zol contains phenol (corrosive liquid/poison) and guanidine thiocyanate (irritant and will cause **BURNS** on skin. Use gloves and eye/face protection (face shield and safety goggles) when using this solution. **In case of contact:** Immediately flush eyes or skin with a large amount of water for at least 15 minutes and seek for medical attention if needed.

Procedures for Isolation of Total RNA from Cells, Tissues, Blood and Body Fluid.

Note: This kit is designed for total or small RNA isolation from up to $5x10^5$ cells and <5mg tissues. For larger samples ($<10^7$ cells and <100mg tissues), please use kit EZCR305. You can also purchase the Mini spin column separately and use the same protocol by doubling the buffers and solutions. All procedures are performed at 4^0C and the 100% ethanol used is pre-warmed to room temperature.

I). Samples Preparation: Sample volume should not exceed 10% of the volume of Column-zol used for homogenization.

1. Monolayers: **recommend to process 10^3 - $1x10^5$**Cells
   The amount of **Column-zol** to be added to the culture plate/dish is based on the area of a culture dish and not on cell number. Use 1ml of the solution per $10cm^2$ of culture dish area. Poor off media and add 200µl reagent per well of a 24-well plate ($2cm^2$) and mix well by pipetting several times to ensure lysis. Incubate the mixture for 5 minutes at room temperature and proceed with the step II.
2. Cell Suspensions: **recommended to process $5x10^3$ - $5x10^5$**Cells
   Pellet cells by centrifugation. Carefully remove the supernatant and add Column-zol reagent for cell lysis. The amount of reagent is based on cell number. Add 0.4ml of Column-zol for up to $5x10^5$ cells and mix well by pipetting several times to ensure lysis. Incubate the mixture for 5 minutes at room temperature and proceed with the step II.

3. Tissues: **recommend <5mg.** Large samples can exceed the binding capacity of the column or increase DNA contamination.
   a. Use 0.4ml of **Column-zol** for up to 5mg of tissue. For processing tissue with high DNA content such as spleen, use 3mg in 0.4ml **Column-zol**. Homogenize tissue samples in a Polytron-type or glass-teflon homogenizer on ice.
   b. This step is required for samples with high content of proteins, fat, polysaccharides or extracellular material such as muscles, fat tissue and tuberous parts of plants. Remove insoluble material by centrifugation at 12,000g for 5 minutes at 4^0C. Transfer the clear supernatant to a fresh tube and proceed with the step II.
4. Liquid samples (body fluids, in vitro enzymatic reactions or labeling cleanup) : **recommended to process 0.1ml of liquid**

   Use 2.5-3 volumes of **Column-zol** reagent per volume of liquid sample. For processing smaller samples, supplement with RNase free water to minimal volume of 0.1ml and proceed with the step II.
   For example: add 0.3ml of **Column-zol** reagent to up to 0.1ml of a liquid sample.
5. Whole blood or plasma sample: recommended up to100µl sample
   a. Add 3 volumes of **Column-zol** reagent to each volume of liquid sample.
   b. Supplement each 100µl sample with 10µl of 5N acetic acid and mix well by vortex.
   c. Incubate at room temperature for 5 min.
   d. Centrifuge to remove particulates at 12,000xg for 3 min and carefully transfer the supernatant to a new RNase-free tube.

II). DNA, protein and polysaccharide precipitation: the amount of RNase-free water added to the sample is based on the initial amount of Column-zol reagent used for homogenization.

a. Add 0.4ml of RNase-free water (to the sample from step I) per 1ml of **Column-zol** used for homogenization. Mix the mixture vigorously for 15 seconds in a leak-prove, DNase/RNase free screw cap micro-centrifuge tube (EZC165SN or EZC200SN) and incubate at room temperature for 15 minutes.
b. Centrifuge the samples at 12,000g for 15 min. For processing the samples with high DNA content, spin at 16,000g for 15min at 4^0C. RNA remains soluble in the supernatant after centrifugation.
c. Transfer 75% of the supernatant (eg. 0.5ml of supernatant when using 0.5ml **Column-zol** reagent at initial step) to a new tube, leaving a layer of the supernatant above the DNA/protein pellet.
   Note: DNA can be recovered from the pellet, please contact us for the protocol

III). Additional extraction with CZ additive: recommended for samples with high content of DNA and/or extracellular material

a. Add 2.5µl (0.5% of the supernatant volume) of **CZ additive** to 0.5ml of transferred supernatant. Shake to mix the tube for 15 seconds and incubate at 4-25^{0}C for 3-5 min and then centrifuge at 12,000g for 10min at same temperature.
b. Transfer the supernatant carefully (without disturbing the lower phase) to a clean tube.

IV). Binding RNA to spin column:

a. Add 1-1.25 volume ethanol (95-100%) to the supernatant obtained in either step II) or III). Mix by inverting the tube several times. Example: add 0.5-0.625ml ethanol to 0.5ml supernatant.
   Note: This step is for recovering total RNA>17nt. To enrich or obtain small RNA in separate fraction, see **Appendix.**
b. Load the mixture to the column with collection tube and spin for 1 min at 4^0C at 12,000xg. Discard the flow-through.
   Note: Maximal loading capacity of the RNA Tini spin column is 350µl. Repeat this step if more than 350µl is processed

V). Wash with 75% ethanol:

a. Add 350µl of 75% ethanol (v/v) and centrifuge for 1 min. Discard the flow-through. Repeat this step.
b. Centrifuge the column for an additional 2 min to remove the excess ethanol.
   Note: this step is important since residual ethanol may affect the downstream applications.

VI). Elute RNA from column:

a. Add 5-20µl of DNase/RNase free water (pre-warmed at 65^{0}C) to the center of the Tini spin column and incubate at room temperature for 5 min. Centrifuge at max speed for 1 min.
b. The eluted RNA can be used immediately or store at -80^{0}C.

Appendix: Enrichment procedure for small RNA (Continue from step **IV**)

a. Add 1/3 volume of 100% ethanol to the supernatant from step II or III (e.g. add 100µl 100% ethanol to 300µl supernatant).
b. Load the mixture to the column containing **new** collection tube and spin for 15 sec at 4^0C at 10,000xg. **SAVE** flow-through. Column can be saved if you want to recover only the large RNA (>200nt) by following the rest of the steps V-VI.
c. Add 2/3 volume of 100%ethanol to the flow-through (e.g. add 200µl ethanol to 300µl flow-through) and mix.
d. Load the mixture into a new spin column with used collection tube and follow the rest of the steps V-VI.

Trouble shooting guide:

Problem Description	Possible cause
Low yield	Incomplete homogenization or lysis of samples
260/280 ratio<1.6	-Insufficient vol. of **reagent** used for homogenization -Acidic water used for the OD measurement -proteoglycan or polysaccharide contamination
RNA degradation	-Tissue were not immediately processed/frozen in liquid N_2 after removal from an animal -samples used for RNA preparation were stored at -20^{0}C instead -80^{0}C -cells were dispersed by trypsin digestion -water, ethanol, tips or tubes used were not RNase free
DNA contamination	-too much tissue or cells were used for the volume of the reagent used in homogenization -Sample used for RNA prep contained organic solvents, high salt or alkaline solution

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