LaboPass™
Labozol Reagent Cat. No. CMRZ001 100 mL

COSMO GENETECH

The LaboPass™ Labozol Reagent is a ready-to-use reagent, designed to isolate total RNA including miRNA from cell and tissue samples of human, animal, plant, yeast and bacterial origin. Labozol Reagent shows superior performance in extraction of total RNA with high integrity due to effective inhibition of RNase by phenol and guanidine thiocyanate, which are the major components in this reagent. The extracted RNA can be used in downstream applications such as cDNA synthesis, Northern blot, mRNA isolation, RNase protection assay and other RNA-based assay.

Storage and Stability

Labozol Reagent is stable for at least 12 months when stored at 4°C.

Caution

Labozol Reagent contains very hazardous and toxic chemicals (Phenol, Guanidine thiocyanate, etc). All works with Labozol Reagent must be performed in a fume hood with wearing gloves. Direct contact with Labozol Reagent to skin, eyes or respiratory tract may cause chemical burns. If contact to skin or eyes occurs, immediately wash with running water for 15 minutes.

Materials required (not provided)

Chloroform
Isopropyl alcohol
75% ethanol in DEPC-treated water
RNase-free water

RNA isolation procedure

Except centrifugation steps, unless otherwise stated, whole isolation procedure should be carried out at room temperature.
(15°C to 30°C)

STEP 1. Sample homogenization

Select optimized disruption or homogenization method depending on the type of sample as stated below. Complete disruption and homogenization is required for getting high yield of RNA.

Animal tissue

a. Excise out and weigh the appropriate amount of tissue from animal or frozen tissue sample.
b. Add 1 ml of Labozol Reagent per 50-100 mg of tissue sample. Using a glass/teflon, rotor-stator or bead mill homogenizer, completely homogenize tissues, if it is required.
c. Incubate the homogenized sample for 5-10 minutes at room temperature.

Plant

Disruption method could be variable depending on the nature of the plant including polysaccharides content and tuberous parts. For complete cellular disruption, enzymatic homogenization is also available in combination with mechanical techniques.

a. Excise out the appropriate amount of tissue from plant sample, and then chop or mash down.
b. Add 1 ml of Labozol Reagent per 50-100 mg of plant sample. Homogenize using a glass/teflon grinder or bead mill homogenizer.
c. Incubate the homogenized sample for 5-10 minutes at room temperature.

Adherent cell

a. Aspirate growth media in culture dish.
b. Add 1 ml of Labozol Reagent per culture dish .
(100 mm, 4 x 10^6-1 x 10^7 cells)
c. Lyse cells by repetitive pipetting up-down until the clumps are disappeared.
d. Incubate the disrupted sample for 5-10 minutes at room temperature.

Suspension cell

a. Collect cells by centrifugation and aspirate out growth media.
b. Immediately add 1 ml of Labozol Reagent per 4 x 10^6 - 1 x 10^7 cells.
c. Lyse cells by repetitive pipetting up-down until the clumps are disappeared.
d. Incubate the disrupted sample for 5-10 minutes at room temperature.

* At this point, the sample can be stored at -70°C for at least 1 month.

(Optional) Removing insoluble material

If sample from STEP 1 contains large amounts of proteins, lipids, polysaccharides or extracellular material, centrifuge it at 12,000 x g for 10 minutes at 4°C. Transfer the clarified homogenate to a new RNase-free tube. Be careful to avoid insoluble pellet and sustained fatty layer.

STEP 2. Phase separation

a. Add 0.2 ml of chloroform per 1 ml Labozol Reagent, and tightly secure the tube.
b. Shake the tube vigorously for 15 seconds to mix sample.
c. Incubate for 3 minutes at room temperature.
d. Centrifuge 12,000 x g for 15 minutes at 4°C.
e. Following centrifugation, three phases were appeared.
 - Upper (Colorless) : Aqueous phase
 - Interphase
 - Lower (Red) : Phenol-chloroform phase
f. Without any disturbing the interphase, transfer carefully the colorless upper aqueous phase which contains RNA to a new RNase-free tube. Do not take the entire aqueous phase to avoid contamination with interphase. Since interphase could contain DNA , lipids, carbohydrates and proteins, contamination with interphase can interfere with downstream applications.

STEP 3. RNA precipitation

a. Add 0.5 ml of isopropyl alcohol per 1 ml of Labozol Reagent.
b. Incubate for 10 minutes at room temperature.
c. Centrifuge 12,000 x g for 10 minutes at 4°C.
d. The RNA will be pelleted with white color on the side and bottom of the tube.
e. Without any disturbing, discard the supernatant.

STEP 4. RNA wash

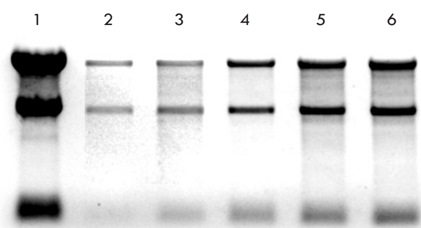
a. To wash pellet, add 1 ml of 75% ethanol per 1 ml of Labozol Reagent.
 * At this point, the sample can be stored at -70 °C for at least 1 month.
b. Vortex sample briefly and then centrifuge at 7,500 x g for 5 minutes at 4°C.
c. Discard supernatant and air-dry the pellet for 5-10 min at room temperature.
 Do not completely dry pellet, which results in lower solubility of pellet.

STEP 5. Resuspension

a. Add appropriate RNase-free water and completely resuspend by pipette up-down.
b. If RNA pellet is not completely dissolved, incubate at 55°C - 60°C for 5-10 minutes.
c. RNA concentration is determined by absorbance at 260 nm on a spectrophotometer.
d. RNA sample can be used in downstream applications or stored at -70 °C.

Gel electrophoresis of total RNA

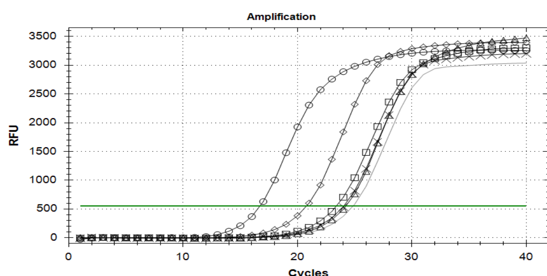
Total RNA was isolated from various cells and tissues with Labozol Reagent and run on denaturing agarose gel electrophoresis.



Lane 1 : HEK293T
Lane 2 : NIH3T3
Lane 3 : mouse brain
Lane 4 : mouse liver
Lane 5 : mouse kidney
Lane 6 : mouse spleen

Application to real-time qRT-PCR

Total RNA extracted with Labozol Reagent can be successfully used in Real-time qRT-PCR assay.



Total RNA extracted from various cultured cells and tissues with Labozol Reagent was reverse-transcribed into cDNA using LaboPass™cDNA synthesis kit (Cat.No. CMRTK001). The resulting cDNA was applied to real-time qPCR for GAPDH mRNA using LaboPass™ SYBR green Q master (Cat.No. CMQS200).
Circle, HEK293T ; triangle, NIH3T3 ; cross, mouse brain ; square, mouse liver ; diamond, mouse kidney ; grey, mouse spleen.

Expected yields

The table below presents typical yields of RNA (A260/280 of >1.8) from various materials.

Material	Quantity	RNA (Yield)
Epitherial cells	1 X 10^6 cells	10 ~ 20 ug
Fibroblast cells	1 X 10^6 cells	7 ~ 8.5 ug
Brain	1 mg	1 ~ 1.4 ug
Spleen	1 mg	3 ~ 4.5 ug
Kidney	1 mg	4 ~ 6 ug
Liver	1 mg	5 ~ 6 ug

Troubleshooting guide

Problem	Cause	Solution
Low yield of RNA	• Incomplete lysis • Final RNA pellet was incompletely redissolved	• Complete homogenization or lysis of sample • Increase the solubilization rate
RNA degradation	• Not immediately processed or frozen after collection	• Sample must be processed or frozen immediately after collection • Add β-mercaptoethanol 10 ul per 1 ml of Labozol Reagent
Contamination of genomic DNA	• The aqueous phase was contaminated with the phenol phase	• Do not attempt to draw off the entire aqueous layer after phase separation

COSMOGENETECH

http://www.cosmogenetech.com
Email : labopass@comsmogenetech.com
Tel 82-2-465-6215 Fax 82-2-465-6278