BioChain®
www.biochain.com
Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

User's Manual and Instructions

Easy-mRNA Kit

Catalog Number: K2011010

Introduction
Easy-mRNA Kit can be used to either isolate mRNA directly from tissues and cells or isolate mRNA from total RNA. You can get the best quality mRNA with this easy to use kit.

Features
- Isolate mRNA from small or large samples
- Isolate mRNA either from tissues or from total RNA
- Isolated mRNA is intact and over 12 kb in length

Application
The Easy-mRNA Kit can be used for isolation of mRNA from animal tissues, plant tissues, cultured cells, yeast and bacteria.

Description
Easy mRNA Kit is composed of two elements: (1) RNAexol I, and RNAexol II, which can be used for isolation of total RNA from a broad spectrum of cell and tissue types such as animal tissue, plant tissue, yeast and bacterial, and (2) mRNA isolation reagents for purification of mRNA from up to 10 mg of total RNA. The isolated mRNA is intact and over 12 kb in length, and can be used for cDNA library construction, subtracted probe generation, RT-PCR, Northern blot analysis, primer extension, RNA protection assay, and In vitro translation.

Storage and Stability
Store the solutions at the appropriate temperature. The kit is stable for one year when handled properly

Component

Reagent	Catalog No.	Quantity	Storage
RNAexol I	K2011010-1	100 ml	4ºC
RNAexol II	K2011010-2	100 ml	4ºC
Solution M	K2011010-3	20 ml	4ºC
RNase Inactive Solution	K2011010-4	10 ml	4ºC
Oligo dT cellulose	K2011010-5	250 mg (25 mg x 10)	-20ºC
Oligo dT buffer	K2011010-6	100 mlx2	4ºC
Wash buffer	K2011010-7	50 ml	4ºC
Elution buffer	K2011010-8	10 ml	4ºC
DEPC treated water	K2011010-9	1.2 ml	4ºC
3M NaAC (pH 4.5)	K2011010-10	1.2 ml	4ºC
2ml microcentrifuge tube with Column	K2011010-11	10 units	Room Temperature
2ml microcentrifuge tube	K2011010-12	10 units	Room Temperature
15ml centrifuge tube	K2011010-13	10 units	Room Temperature

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Total RNA Isolation

Brief Protocol

1. Homogenization: 1 ml RNAexol I and II/10-100 mg tissues, or 5-10 x10^6 cells
2. RNA extraction: 0.4 vol. of Chloroform/1 vol. of Homogenate.
3. RNA precipitation: 1 vol. of isopropanol /1 vol. of aqueous phase
4. RNA wash: 75% ethanol soak for 10 minutes

Detailed Protocol

2.1 Homogenization:

a. Tissues:

Homogenize 1g of fresh or frozen tissue sample with 10 ml RNAexol I in hand-held glass-Teflon or polytron homogenizer till no visible slices.

b. Cells:

Homogenize 5-10^6 cells by adding 1 ml RNAexol I directly on the cell culture dishes or flasks, and stay at room temperature for 5-15 minutes.

2.2 Following homogenization, add equal volume RNAexol II, and shake vigorously.

2.3 RNA Extraction:

Add 0.4 volume of chloroform for 1 volume of RNAexol I, cover the samples tightly, shake vigorously for 15 seconds and let them stay on ice or at 4°C for 10 minutes. Centrifuge the homogenate at 12,000 rpm 4°C for 15 minutes.

2.4 RNA precipitation:

Carefully transfer the aqueous phase to a fresh tube and mix with equal volume of isopropanol. Store the samples at -20°C for 20 minutes. Then centrifuge at 12,000 rpm 4°C for 15 minutes. RNA pellet (often invisible before centrifugation) is at the bottom of the tube.

2.5 Remove the supernatant and dissolve RNA in appropriate volume of Solution M. Add equal volume of isopropanol, mix well, store at -20°C for 20 minutes. Then centrifuge at 12,000 rpm 4°C for 15 minutes.

2.6 RNA Wash:

Remove the supernatant and carefully wash RNA pellet with equal volume of RNAexol I 75% ethanol. Then replace 75% ethanol with the same volume, break the pellet as possible as you can and soak the RNA pellet at room temperature for 10 minutes.

2.7 Centrifuge at 12,000 rpm for 10 minutes to collect RNA. Dry the pellet under a vacuum for 5-10 minutes (don't make the pellet completely dry!). Dissolve the RNA pellet in supplied RNase Inactive Solution (If total RNA will be used for mRNA isolation) or DEPC treated water. Try your best to make RNA concentration >3.3 μg/μl.

Expected yield of total RNA

a. Tissues (μg/100 mg tissue)	400-600 μg	liver
	100-200 μg	kidney
	30-50 μg	skeletal muscle, brain
	100-200 μg	placenta
b. Cultured cells (μg/10^6 cells)	20 μg	epithelial cells
	8 μg	fibroblasts

The final preparation of undegraded RNA is free of DNA and protein and has an 260/280 ratio=1.8-2.0

Special Handling Precautions

The RNAexol reagent contains poison and irritant (phenol and guanidine salts). Use gloves and eye protection (shield, safety goggles). Do not get in skin and clothes, and avoid breathing vapor. In case of contact:

immediately flush with a large amount of saline or water for at least 15 minutes and seeks immediate medical attention.

mRNA Isolation

Following procedure is for isolation of mRNA from 1 mg total RNA.

I. Oligo-dT cellulose treatment

1. Put one tube of oligo-dT cellulose (25 mg) to a RNase free centrifuge tube.
2. Add 5 ml oligo dT buffer suspend and soak oligo-dT cellulose for 10 minutes. (It is better to rotate the mixture at room temperature for 10 minutes)
3. Spin at 2,000 rpm room temperature for 5 minutes and discard the supernatant.
4. Add 5 ml oligo-dT buffer resuspend oligo-dT cellulose.

Note: Oligo dT buffer may have precipitation, warm up the buffer at 65°C for 10 minutes if there is precipitation in the buffer

II. Denaturing RNA

After isolation of total RNA with RNAexol supplied in the kit, total RNA is dissolved either in RNase Inactive Solution or DEPC treated H_2O. Please make RNA denaturing solution according to table below:

RNA dissolved in	Bring volume to 925 µl by adding
RNase Inactive Solution 0.3 ml, concentration ≤ 3.3µg/µl	DEPC treated H_2O 0.625 ml
DEPC treated H_2O 0.625 ml, concentration ≤ 1.6 µg/µl	RNase Inhibitor Solution 0.3 ml

The ratio of RNase Inactive Solution to DEPC treated H_2O should be kept at 3:6.25. Mix well and heat RNA denaturing solution at 65°C for 10 minutes.

III. RNA Binding

1. Transfer RNA mixture to oligo dT cellulose tube in **step I, 4**. Mix well and rotate the mixture at room temperature for 15-30 minutes.
2. Spin at 2,000 rpm room temperature for 5 minutes and discard supernatant.
3. Preheat Elution buffer at 65°C for step V.

IV. Wash

1. Add 5 ml oligo dT buffer to resuspend oligo-dT cellulose.
2. Spin at 2,000 rpm room temperature for 5 minutes and discard the supernatant.
3. Repeat step 1 and 2 for one time.
4. Add wash buffer 3 ml to resuspend oligo dT, spin at 2,000 rpm room temperature for 5 minutes and discard the supernatant.
5. Resuspend the oligo-dT cellulose with 700 µl wash buffer and transfer to a column, spin at 12,000 rpm for one minute.
6. Discard wash buffer and spin at 12,000 rpm for another one minute.

V. Elution

1. Transfer the column to a new 2 ml microcentrifuge tube.
2. Resuspend the oligo-dT cellulose with 500 µl of Elution buffer (preheated to 65°C at **step III.3**).
3. Spin at 12,000 rpm for one minute.

VI. Precipitation

1. Add 0.1 volume 3M NaAc (pH 5.2), 2-3 volume 100% ethanol to the 2 ml tube which contains mRNA, mix well and keep on dry ice for 30 minutes.
2. Spin at 12,000 rpm 4°C for 20 minutes, RNA pellet should be visible at the bottom of the tube. Discard the supernatant.
3. Wash mRNA with 1.5 ml 75% ethanol. Spin at 12,000 rpm 4°C for 10 minutes.
4. Aspire the supernatant carefully. Do not lose mRNA pellet.
5. Air dry the pellet and dissolve it with DEPC treated water or appropriate buffer.