

AutoGenprep 965

Application Guide

RNA Animal Tissue / Cultured Cells



I. **Purpose:** To extract total RNA from animal tissue or cultured cells

II. **Chemical Principal:** Modified Acid GTC extraction method.

III. **Pretreatment of Tissue Samples:**

A. **Animal Tissue**

1. Place an appropriate weight* of tissue samples and add TR-R0 (Tissue Homogenization Solution) at 1ml per 50mg of tissue.
2. Homogenize the samples completely on the AutoDisrupter24 or another homogenizer.
3. Transfer 0.5ml of homogenized samples onto a 96 deep-well plate.
4. Run Standard parameter file of AutoGenprep965 Tissue RNA protocol

B. **Cultured Cell**

1. Cultivate cells (BALB/3T3) with DMEM medium containing 10% FBS, peel the cultivated cells with 0.1% Trypsin, and count the number of cells.
2. Collect the cells by centrifugation and waste supernatant. Resuspend the collected cells by tapping and lyse the cells with TR-R0 (Tissue Homogenization Solution).
3. Adjust the cell concentration of the lysate to 500,000cells / 0.5ml of lysate with TR-R0 (Tissue Homogenization Solution).
4. Transfer 0.5ml of the pre-adjusted cell concentration lysate into 96 deep well plates.

IV. **Protocol Parameters:**

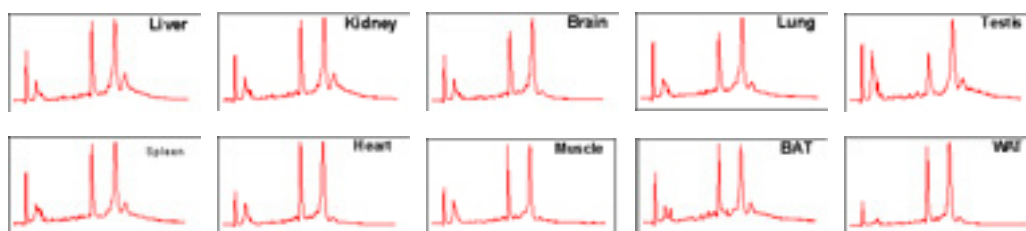
A. **Sample Volume:** 0.5ml of homogenized lysate (equivalent to 10-50mg of tissue)

B. **Maximum Number of Samples:** Up to 384 (4 of 96 plates) samples per run.

C. **Processing Time:** 2.8 hours for 192 samples
4.7 hours for 384 samples.
This includes a 20 minute DNA drying time

D. Yield and Purity:

Sample Type	Optimal weight range (mg)	Max. weight (mg)	RNA Yield $\mu\text{g}/\text{mg}$ tissue	Purity OD260/280
Liver	5 ~ 50	50	4.4	2.0
Kidney	5 ~ 75	75	2.4	2.0
Brain	10 ~ 75	75	0.9	2.0
Lung	10 ~ 75	75	1.1	2.0
Testis	10 ~ 75	75	1.5	2.0
Spleen	5 ~ 50	50	3.8	2.0
Heart	10 ~ 40	40	0.7	2.1
Muscle	10 ~ 40	40	0.3	2.0
Brown Adipose (BAT)	10 ~ 75	75	0.3	2.0
White Adipose (WAT)	10 ~ 150	150	0.1	1.9
BALB/3T3 cell	0.5 million	N/A	12/million	1.9

E. Quality of extracted total RNA:**V. Extraction and Purification Process**

Process Site	Purpose	System Process
1. Manual	Homogenize tissues	Add Reagent TR-R0 and homogenize samples.
2. Automated	Remove protein and cellular debris	Add Reagent TR-R1 and TR-R2, mix, centrifuge to pellet debris and transfer supernatant to new deep-well plate.
3. Automated	Precipitate RNA	Add Reagent TR-R4, mix, precipitate RNA, centrifuge and discard supernatant.
4. Automated	Wash RNA	Add Reagent TR-R5/6, mix, centrifuge, and discard supernatant, repeat.
5. Automated	Dry RNA	Add Reagent TR-R7, mix, centrifuge, discard supernatant, and centrifuge.
6. Automated	Resuspend RNA	Add an appropriate resuspension buffer* and mix.

* *An appropriate RNA resuspension buffer should be prepared on the reagent reservoir No.9 for automated dispensing*

AutoGen

84 October Hill Road, Holliston, MA 01746

Tel: 800-292-5678 or 508-429-5965 | Fax: 508-429-9765

E-mail: dna@autogen.com