NA-24T RNA protocol

miRNA highly contained total RNA isolation from mouse brain with NA-24T

Introduction

MicroRNAs (miRNAs) play important regulatory roles in biological processes such as development, differentiation and defense against infection by targeting mRNA for cleavage or translational repression. To identify which miRNA involves with biological phenomena, many researchers are analyzing expression levels of miRNAs. In this note, we performed real-time RT-PCR for total RNA samples isolated using NA-24T, an AGPC (acid guanidine phenol chloroform) reagent kit or silica solid-phase extraction kit to detect a miRNA (miR-16) in each RNA sample.

Methods

Isolation of total RNA: Total RNA was isolated from a frozen mouse brain tissue in the following method respectively.

1. NA-24T: RNA protocol (default parameter setting), 50mg of tissue, dissolution volume 50µl
2. AGPC reagent kit: according to the product protocol, 50mg of tissue, dissolution volume 50µl
3. Silica Solid-phase based column kit: according to the product protocol, 30mg of tissue, elution volume 30µl x 2 (total 60µl)

Yield calculation: Absorbance of 230nm, 260nm and 280nm was measured for each RNA solution by spectrometer. RNA yield was calculated as follows: A260 X 40 X dilution factor X final volume.
Bioanalyzer analysis: RNA was analysed according to the Agilent Bioanalyzer manufacturer's instruction.

Measurement of miRNA miR-16:
Real-time RT-PCR was performed using the following reagent kit and instruments.
Assay kit: TaqMan® MicroRNA Reverse Transcription (RT) Kit（ABI, 4366596）
TaqMan® MicroRNA Assay ABM 000008 hsa-miR-16(ABI,4365746)
TaqMan Universal MasterMix No AmpErase UNG (ABI, 4324018)
Reverse transcription: TaKaRa Thermal Cycler Mp
Real-time PCR: ABI PRISM® 7000 Sequence Detection System

Measurement of GAPDH mRNA:
Real-time RT-PCR was performed using the following reagent kit and instruments.
Assay kit: ABI PRISM® Rodent GAPDH Control Reagents VIC Probe (ABI,4308313) and One-step RT-PCR Master Mix Reagents (ABI, 4309169).
RT reaction and real-time PCR: ABI PRISM® 7000 Sequence Detection System

Starting materials: For each isolation method or each RNA molecule, 25ng, 2.5ng or 0.25ng of total RNA was used in duplicate (n=2)

Results

Total RNA yield and quality

Table1. Analysis of the yields and purity of RNA isolated using NA-24T.

Isolation method	Yield (µg/mg tissue)	Purity (A260/280)	Purity (A230/260)
1. NA-24T	0.95	1.91	0.49
2. AGPC reagent kit	1.28	1.95	0.53
3. Silica solid-phase kit	0.72	2.19	0.56

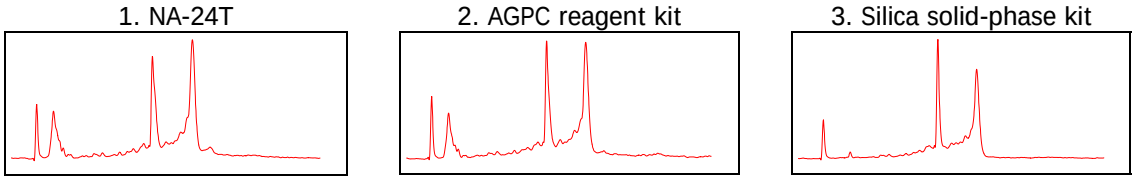
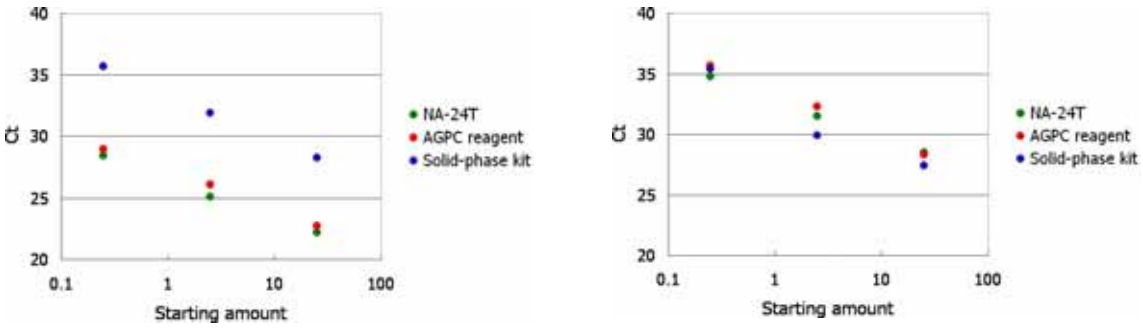


Fig1. Bioanalyser analysis of total RNA

Analysis of miRNA and mRNA

Table2. Threshold cycle (Ct) number at real-time PCR for miR-16 and GAPDH in total RNA isolated with NA-24T and other methods.

miRNA/gene	Isolation method	Ct		
		Starting amount		
		25ng	2.5ng	0.25ng
miR-16	1. NA-24T	22.2	25.1	28.4
	2. AGPC reagent kit	22.7	26.1	28.9
	3. Silica solid-phase kit	28.2	31.9	35.7
GAPDH	1. NA-24T	28.5	31.5	34.8
	2. AGPC reagent kit	28.3	32.3	35.7
	3. Silica solid-phase kit	27.4	29.9	35.4



miR-16 GAPDH

Fig2. Plot between the Ct and the starting amount of total RNA.

The Ct values for the RNA samples with NA-24T and AGPC reagent kit were lower by about 6 cycles than that one for silica solid-phase kit. It means that the amount of miRNA in the total RNA isolated with NA-24T or AGPC reagent kit is more by 60 times than in that with silica-phase kit.
The Ct values for GAPDH mRNA were approximately same.

NA-24T isolates total RNA which is good enough for the analysis of expression levels of both miRNA and mRNA.

KURABO INDUSTRIES LTD.
Bio-Medical Department
4-31, 2-chome, Kyutaro-machi, Chuo-ku
Osaka, 541-8581 Japan
Phone: 81-6-6266-5010
Fax: 81-6-6266-5011
bio@ad.kurabo.co.jp