



Application

# RNA extraction from brown adipose tissue (example)

Product

FastGene® RNA Basic Kit (FG-80050, FG-80250)

Manufacturer

NIPPON Genetics EUROPE GmbH

The following data was kindly provided by a Japanese customer.

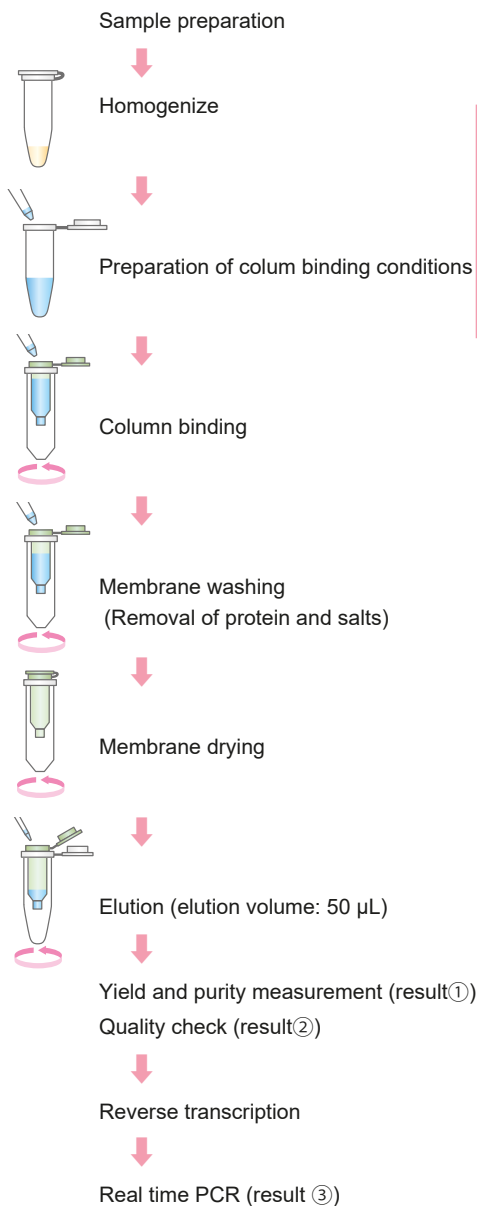
Points of this application note

In RNA purification, it is important that the homogenisation is completely carried out in order to purify a good quality and amount of RNA which is required for downstream application.

In a general RNA purification kit, homogenization using lysis buffer is performed not only to physically break up cells and tissues, but also to inactivate RNase contained in cells. Especially in tissues and cells containing a large amount of RNase such as adipose tissue, the fast inactivation of RNase by homogenization is a key point in the purification of high-quality RNA. As an application example of FastGene® RNA Kit, we introduce RNA purification using adipose tissue.



Workflow



Experimental conditions

- Sample information:  
Mouse scapular inter-brown adipose tissue 30 -50 mg

Here is the point!

- Homogenization condition:  
RL buffer 600 µL+1 M DTT 40 µL. Add tissue pieces.  
↓  
Homogenize with an electric homogenizer (centrifuge at 17,700 x g for 5 min)  
↓  
Approximately 400 µL of supernatant was separated and mixed with 400 µL 70% EtOH  
※ Be careful not to take up fat when collecting the supernatant!

- Yield and purity measurement  
Device: SimpliNano (Biochrom)
- Quality check:  
Reagents: Agilent RNA 6000 Nano Chips  
Device: Agilent 2100 Bioanalyzer
- Reverse transcription:  
Input RNA amount: 0.5 µg (10 µL per reaction)  
Reverse Transcriptase: ReverTra Ace® qPCR RT Master Mix with gDNA Remover (Code No. FSQ-301)
- Real time PCR conditions:  
Input cDNA amount: 5 µL of 200-fold dilution (15 µL per reaction)  
Reagent: KAPA SYBR Fast qPCR kit (Universal qPCR kit)  
Apparatus: LightCycler 96

• Reaction composition		
KAPA SYBR FAST qPCR Master Mix (2X)		7.5 µL
10 µM forward primer		0.5 µL
10 µM reverse primer		0.5 µL
Template DNA		5 µL
MQ water		1.5 µL
<hr/>		
total		15 µL

- Program

Initial denature	95 °C	10 min	
Denature	95 °C	10 sec	} 45 cycles
Annealing	60 °C	10 sec	
Extension	72 °C	10 sec	
Melting			






## Results

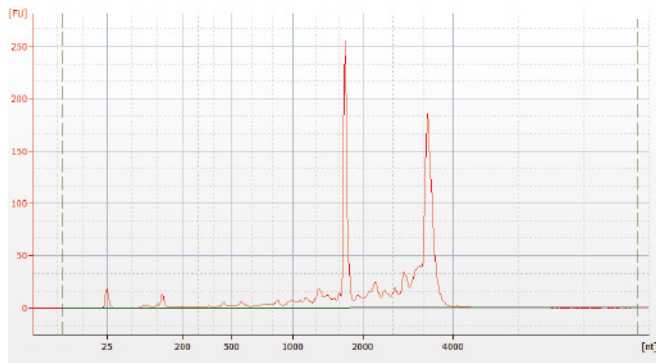
### Result ① Yield and purity measurement

Sample	Elution volume	concentration	A <sub>260</sub> /A <sub>280</sub>	A <sub>260</sub> /A <sub>230</sub>	A <sub>260</sub>	A <sub>280</sub>	A <sub>230</sub>
1	50 µL	392.3 ng/µL	2.08	2.28	9.84	4.76	4.33
2		305.2 ng/µL	2.11	2.26	7.70	3.69	3.45
3		196.2 ng/µL	2.07	2.24	5.04	2.50	2.33

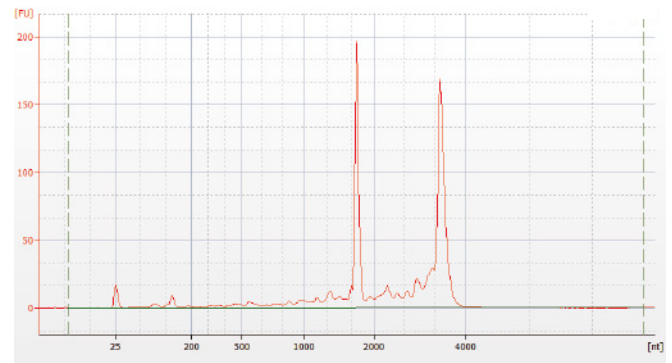
### Result ② Quality check (RIN value, 28S/18S)

Sample	RIN value	28S/18S
1	8.6	1.4
2	8.8	1.5
3	8.9	1.5

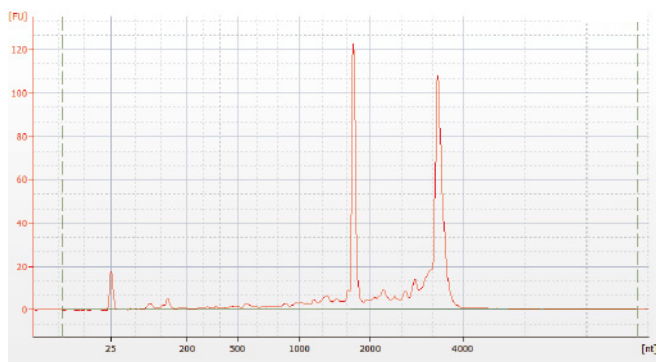
#### Sample 1



#### Sample 2

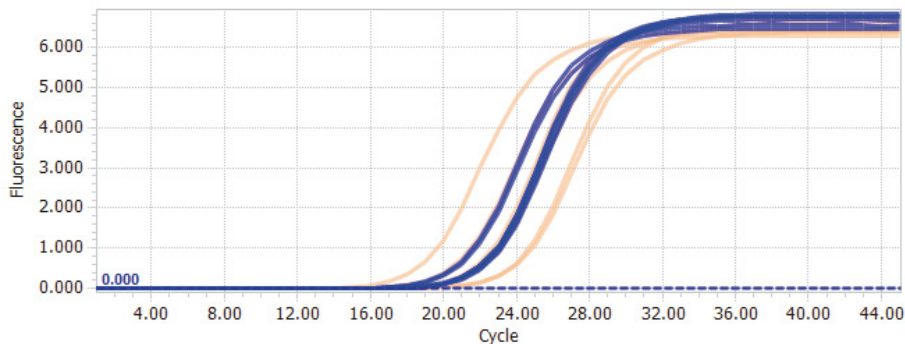


#### Sample 3



RNA from brown adipose tissue was obtained with sufficient yield and purity. Also, the results of real-time PCR were as expected.

### Result ③ Real-time PCR



— Sample  
— Calibrating curve

Sample	C <sub>q</sub>	C <sub>q</sub> Mean
1	19.24	19.36
	19.47	
2	21.05	20.94
	20.83	
3	20.59	20.66
	20.73	



Compared to the method using phenol and guanidine thiocyanate, it is easy to isolate high purity RNA in sufficient quantity.

Customer's comment