NIPPON Genetics EUROPE

Application

Performance analysis of “RNA extraction” kits and ”reverse transcription reaction” from mouse liver

Category

Nucleic acid purification / cleanup, reverse transcriptase, qPCR reagent

Product

- RNA extraction

High Pure RNA Tissue Kit（50 rxn）(12033674001) / Roche Diagnostics K.K
FastGene® RNA Basic Kit（FG-80250) / NIPPON Genetics EUROPE GmbH

- Reverse transcription reaction

Transcriptor Universal cDNA Master（05893151001) / Roche Diagnostics K.K
FastGene® Scriptase Ⅱ Master Mix（5X）(LS64) / NIPPON Genetics EUROPE GmbH
FastGene® Scriptase Basic cDNA Synthesis（LS62) / NIPPON Genetics EUROPE GmbH

- qPCR

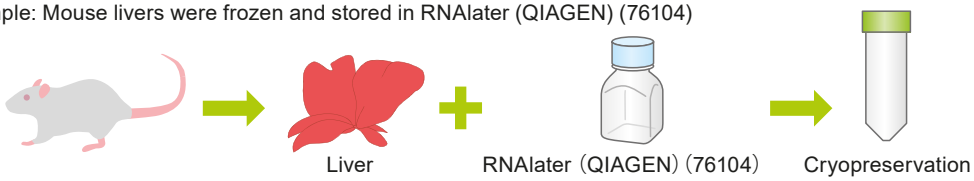
FastStart Essential DNA Green Master Mix（06402712001) / Roche Diagnostics K.K

The following data has been posted with the kindness of customers in Japan.

Outline of the experiment

Similar to existing kits (Roche products), FastGene® products (Scriptase II, Basic) could be used almost equally.

Sample: Mouse livers were frozen and stored in RNAlater (QIAGEN) (76104)



Frozen for about 2 months

Sample collection
Less than 10 mg of tissue

RNA extraction

Absorbance measurement
Nano Drop™

RT reaction

qPCR Cq value measurement
（Light Cycler 96）

RNA extraction

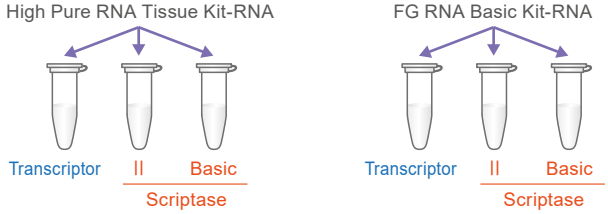
Two types of kits used
- High Pure RNA Tissue Kit (50 rxn) (12033674001)
- FastGene® RNA Basic Kit (FG-80250) + DNase I set (FG-81DN250)
  -” Option: Use on-column DNase I treatment ⇒ Details on next page

High Pure RNA Tissue Kit　FastGene® RNA Basic Kit

RT reaction

Three types of kits used
- Transcriptor Universal cDNA Master (05893151001)
- FastGene® Scriptase II Master Mix (5X) (LS64)
- FastGene® Scriptase Basic cDNA Synthesis (LS62)

Reverse transcription using the above three kits



qPCR

One kit used
- FastStart Essential DNA Green Master Mix（06402712001）

■ Combination table of RNA extraction kit and RT reaction kit

		Reverse transcription reaction		
		Transcriptor Universal cDNA Master	FastGene® Scriptase II Master Mix	FastGene® Scriptase Basic
RNA extraction	High Pure RNA Tissue Kit	1 sample	1 sample	1 sample
	FastGene® RNA Basic Kit ＋ DNase I	1 sample	1 sample	1 sample

For qPCR, three replicates per sample were used.

Experimental conditions

■ RNA extraction

High Pure RNA Tissue Kit: Compliant with the recommended protocol in the High Pure RNA Tissue Kit manual

FastGene® RNA Basic Kit ＋ DNase I set: Instructions for FastGene® RNA Basic Kit

" Optional: On-column DNase I treatment（※）" was added to standard operation.

100 µl of Elution Buffer was used, instead of 50 µl.

（※）Washing procedure with on-column DNase I treatment (Manual on request)

S1 Membrane wash (Protein removal)		300 µl RW1 buffer ≥ 10,000 x g (RT: 20 ～ 25℃) 30 s Transfer the column to a new collection tube (2.0 ml)
S2 DNase I reaction		70 µl DNase I reaction solution (Note: added to the centre of the membrane). Incubation: (RT: 20 ～ 25℃) 10 min
S3 Membrane wash (Enzyme removal		300 µL RW1 buffer ≥ 10,000 x g (RT: 20 ～ 25℃) 30 s After discarding the filtrate, return the column to the collection tube
S4 Membrane wash (Salt removal)		700 µl of buffer RW2 ≥ 10,000 x g (RT: 20 ～ 25℃) 30 s Transfer the column to a new collection tube (2.0 ml)

■ RNA measurement equipment

Absorbance measuring instrument: Nano Drop™

■ Reverse transcription reaction

All kits conformed to the recommended protocols in their respective instructions.

■ qPCR program

qPCR equipment: Light Cycler 96

Preincubation：95℃ 600 s

↓

95℃ 15 s

↓] 45 cycles

60℃ 60 s

↓

Melting：95℃ 1 s → 57℃ 15 s → 98℃ 1 s

↓

Cooling 40℃ 30s

Result

● RNA yield and purity results (absorbance measurement-NanoDrop™)

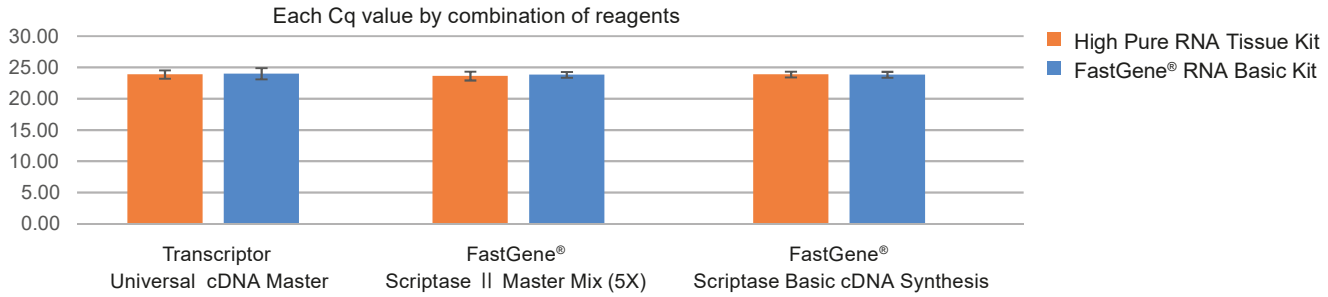
	Sample No.	A230	A260	A280	Yield (ng/μL)	Average yield
High Pure RNA Tissue Kit	1	4.93	10.10	4.82	403.89	472.46
	2	5.03	9.63	4.61	385.32	
	3	7.87	15.70	7.47	628.16	
FastGene® RNA Basic Kit ＋ DNase I	1	5.11	10.52	5.04	420.91	477.36
	2	4.89	9.97	4.77	398.71	
	3	8.44	15.31	7.26	612.46	

There was no significant difference between the reagents, and almost the same results were obtained.

● qPCR result (confirmation of Cq value)

RNA extraction	Reverse transcription reaction	Cq value	mean	stdev
High Pure RNA Tissue Kit	Transcriptor Universal cDNA Master	24.43 23.14 24.09	23.89	0.67
	FastGene® Scriptase II Master Mix (5X)	22.82 24.04 24.03	23.63	0.70
	FastGene® Scriptase Basic cDNA Synthesis	24.02 23.36 24.24	23.87	0.46
FastGene® RNA Basic Kit	Transcriptor Universal cDNA Master	24.83 23.03 24.12	23.99	0.91
	FastGene® Scriptase II Master Mix (5X)	23.29 24.09 24.11	23.83	0.47
	FastGene® Scriptase Basic cDNA Synthesis	24.00 23.31 24.20	23.84	0.47

PCR was performed in triplicate for each sample (cDNA)



There was no significant difference between the reagents and almost the same results were obtained.

Customer's comment

Compared to the products used so far, it was possible to obtain data comparable to the products used, and the price was cheap and very easy to use. I want to use it regularly.

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