Extraction of total RNA from formalin-fixed paraffin-embedded (FFPE) samples

Masaharu Gunji
Nagoya First Hospital, Aichi Medical Center, Japanese Red Cross Society

Precision System Science Co., Ltd.

〈Introduction〉

Determining mutations in oncogenes is imperative to evaluate selective medication in developing and practical use of targeted molecular drugs for cancer treatment. Most of these experiments are carried out using FFPE samples. The accuracy of genetic testing may be affected by the quality of nucleic acid (DNA and RNA) extraction and purification. In addition, biopsy materials represent small pieces of tissue, making efficient recovery of nucleic acids also important. This report describes the use of magLEAD 12gC, a fully automated nucleic acid extraction system manufactured by Precision System Science Co., Ltd. (hereafter PSS), and prepacked reagent MagDEA Dx SV RNA for extraction of total RNA from FFPE samples.

〈Evaluation method〉

Nine specimens of FFPE lung were pretreated (deparaffinization, decrosslinking) as described below. Total RNA extractions were performed from pretreated samples using magLEAD 12gC and MagDEA Dx SV RNA reagents. The quality of the extracted nucleic acids was evaluated by the following criteria:

Quality Evaluation Criteria	Equipment used
Total RNA yield assessment (yield)	Qubit® Fluorometer
Total RNA purity assessment (A260/A280, A260/A230)	Nanodrop™
Total RNA Fragmentation assessment (RIN^e, DV_{200})	TapeStation

〈Pre-treatment method (deparaffinization, decrosslinking)〉

1. Remove tissue sections (10 μm thick) from a glass slide using a scalpel blade.and collect them into a 1.5 mL tube.
2. Add 1 mL of xylene (or suitable alternatives) to the tube containing the tissue section and vortex.
3. Centrifuge at maximum speed for 2 minutes (25℃).
4. Remove the supernatant. Take care not to aspirate the pellet.
5. Add 1 mL of ethanol (96-100%) and vortex.
6. Centrifuge at maximum speed for 2 minutes (25℃).
7. Remove the supernatant. Take care not to aspirate the pellet.
8. Leave the tube open for about 10 minutes
9. Add 180 μL of Prep Buffer B and 20 μL of Proteinase K and vortex.
10. Incubate at 56℃ for 1 h
11. Incubate at 80℃ for 1 h
    ※ If there is only one incubator, leave the sample at room temperature until the incubator has reached 80℃.
12. Centrifuge at maximum speed for 2 minutes (25℃).
13. Collect approximately 200 μL of supernatant into 1.5 mL tubes (magLEAD consumable).

〈Results: Total RNA yield〉

The yield of the extracted total RNA was calculated from concentration results measured with the Qubit RNA BR Assay. Yields of total RNA obtained tended to be higher than samples processed with other manufacturers' reagents (**Figure 1**).

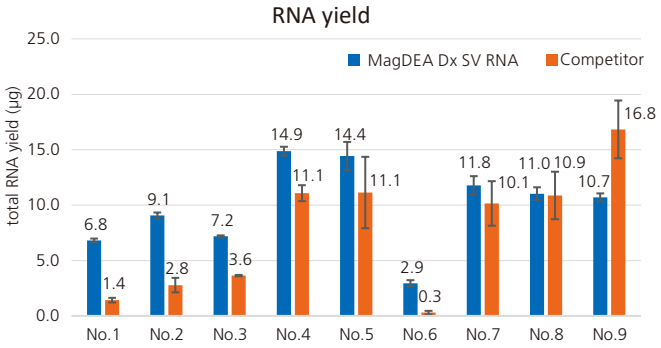

Figure 1 : Comparison of total RNA yields obtained with MagDEA Dx SV RNA and X

〈Results: Total RNA purity〉

The purity of the extracted total RNA was determined on a Nanodrop device. Although the A260/A280 ratio was comparable to results obtained with other manufacturers' reagents, the A260/A230 ratio tended to be lower (**Table 1**).

Table 1 : Comparison of total RNA purity obtained with MagDEA Dx SV RNA and X

	A260/A280		A260/A230	
sample	MagDEA Dx SV RNA	Competitor	MagDEA Dx SV RNA	Competitor
No.1	1.81	1.89	0.99	1.22
No.2	1.89	1.96	1.25	1.61
No.3	1.83	1.93	1.06	1.46
No.4	1.92	1.97	1.37	1.71
No.5	1.94	2.00	1.35	1.67
No.6	1.75	1.53	0.91	0.76
No.7	1.87	1.99	1.23	1.80
No.8	1.90	2.01	1.28	1.88
No.9	1.90	1.98	1.32	1.78

〈Results:Total RNA fragmentation〉

The extent of fragmentation of the extracted total RNA was determined on a TapeStation device. Compared with RNA obtained by extraction with other reagents, the RNA Integrity Number equivalent (RIN^e) and DV_{200} quality metric tended to be higher (**Figure 2, Figure 3**).

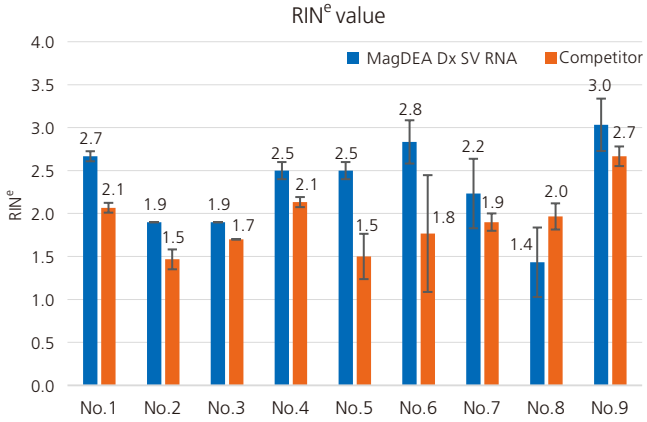

Figure 2 : Comparison of total RNA RIN^e values obtained with MagDEA Dx SV RNA and X

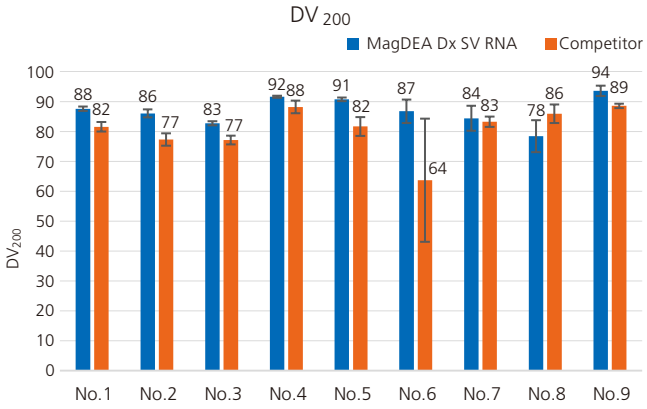

Figure 3 : Comparison of total RNA DV_{200} values obtained with MagDEA Dx SV RNA and X

〈Conclusion〉

The fully automated nucleic acid extraction system "magLEAD 12gC," and the prepacked reagent "MagDEA Dx SV RNA" from PSS enabled total RNA extraction from FFPE samples. Both the quality and yield of the obtained total RNA were comparable or better than those obtained with a competitor method and were considered suitable for mutation analysis of oncogenes.

〈Product Information〉

Product name	Product code
MagDEA Dx SV RNA	E1330
DNase I (Lyophilized)	E1331
Prep Buffer B	E1401
Proteinase K	E1402
magLEAD 6gC	A1060
magLEAD 12gC	A1120
magLEAD Consumable Kit	F4430

Dedicated protocol

Protocol (IC card)	Product code	Notes
MagDEA Dx SV RNA	I7906	magLEAD 6gC IC Card
	I7912	magLEAD 12gC IC Card

Contact details

● For customers in Asia / Pacific
Precision System Science Co., Ltd.
URL: http://www.pss.co.jp
e-mail: service@pss.co.jp

● For customers in North / South America
Precision System Science USA, Inc
URL: http://www.pssbio.com
e-mail: contact@pssbio.com

● For customers in Europe / Africa / Middle East
Precision System Science Europe GmbH
e-mail: contact-psse@pss.co.jp