

# High quality RNA isolation using E.Z.N.A.™ RNA system

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Isolation of high quality RNA is the first and often most critical step in performing many fundamental molecular biology experiments including Northern analysis, nuclease protection assays, RT-PCR®, real-time RT-PCR® and micro-array analysis. There are three major techniques used extensively for RNA extraction: Organic extraction such as Phenol-Guanidine Isothiocyanate (GITC) based solution; Silica-membrane based spin column technology; Paramagnetic particles technology. One of the most commonly used methods is Phenol-Guanidine Isothiocyanate (GITC) based organic extraction, but RNA samples isolated in this way are often contaminated with proteins and other cellular materials, organic solvents such as phenol-chloroform, salts and ethanol. Silica column and paramagnetic particles based RNA isolation systems do not require the use of toxic organic solvents, are relatively simple and efficient, and yield total intact RNA with low level contamination from proteins and cellular materials. However, those methods can often result in significant level of genomic DNA contamination. Omega Bio-Tek has developed diversified RNA isolation kits that cover almost all types of biological sample sources (see product list on page 38) with technologies that effectively reduce the genomic DNA contamination. In this paper, we report the test results from 4 commonly used E.Z.N.A.™ RNA isolation kits to demonstrate the quality of RNA from different sample sources.

## Materials and methods

Samples were collected from field or obtained from commercial sources. Qitec Biotech Co. Ltd supplied all the reagents except reagents from kits.

## RNA extraction

RNA extractions were performed by following user instructions in each kit.

## Real Time RT-PCR®

Real-time RT-PCR® analysis was performed using the Rotor-Gene 3000 (Corbett Life Science). 1 µg of purified RNA was reversed transcribed using 1 µg of random hexanucleotidic primers, 0,5 mM dNTP and 200 U M-MLV Reverse Transcriptase (Promega) at 37 °C for 1 hour in appropriate buffer. SYBR Green Realtime PCR® Master Mix

Source	No. cell mg tissue	A260	A280	Yield (µg)
Cos-7 cell	5 x 10 <sup>6</sup>	0,8653	0,4323	70
Hela cell	5 x 10 <sup>6</sup>	0,7532	0,3710	60
LMH	5 x 10 <sup>6</sup>	0,6353	0,3100	50
Mouse Liver	15 mg	0,7455	0,3826	60
Mouse Spleen	15 mg	0,5368	0,2755	40

**Table 1.** Average yields of total RNA isolated from a variety of cells and tissues using E.Z.N.A.™ Total RNA Kit

	mg tissue	A260	A280	Yield (µg)
Maize Leaves	100	0,5120	0,2561	40
Tomato leaves	100	0,8750	0,4419	70
Paddle leaves	100	0,3752	0,1923	30
Maize seeds	100	0,2512	0,1275	20
Paddle seeds	100	0,1625	0,0850	13

**Table 2.** Average yields of total RNA isolated from a variety of plant using E.Z.N.A.™ Plant RNA kit.

(Toyobo) was used for real-time monitoring of amplification according to the protocol. The real-time PCR® assay was carried out in a volume of 20 µl, 2 µM of each β-actin primer, 1 µl of template cDNA and Master Mix. Thermal cycling conditions were as follows: 95 °C for 1min, 35 cycles of 95 °C for 15s and 55 °C for 15s, 72 °C for 35s and 85 °C for 15s. Accurate amplification of the target amplicon was checked by performing a melting curve.

## Results

We have successfully isolated RNA from a variety samples using our four RNA isolation kits (E.Z.N.A.™ Total RNA Kit I, E.Z.N.A.™

Plant RNA kit, E.Z.N.A.™ Total RNA Kit II and E.Z.N.A.™ HP Total RNA Kit) and also demonstrated that the genomic DNA contamination could be effectively eliminated using on-membrane DNase I digestion or E.Z.N.A.™ HP Total RNA protocol.

## E.Z.N.A.™ Total RNA Kit I (R6834)

The E.Z.N.A.™ Total RNA Kit use silica-membrane technology to isolate high quality of RNA from 30 mg soft animal tissue or 1 x 10<sup>7</sup> Cells in 20 minutes. Briefly, culture cells and tissues are lysed and homogenised in TRK Lysis Buffer (a highly denaturing guanidine isothiocyanate(GITC)-containing buffer). Ethanol is added to provide appropriate binding conditions, and the sample is then applied to an HiBind RNA column. The column is then washed by Wash Buffer I and II. High-quality RNA is then eluted in 50 µl of DEPC water. Figure 1 demonstrates that high quality RNA can be successfully isolated from selected samples with typical yields shown in Table 1. Amplification results from real-time PCR® are shown in Figure 2.

## E.Z.N.A.™ Plant RNA Kit (R6827)

The E.Z.N.A.™ Plant RNA Kit is designed to isolate RNA from variety of plant species by using silica-membrane technology. Figure 3 demonstrates the high quality RNA successfully isolated from various samples and the typical yields are shown in Table 2.

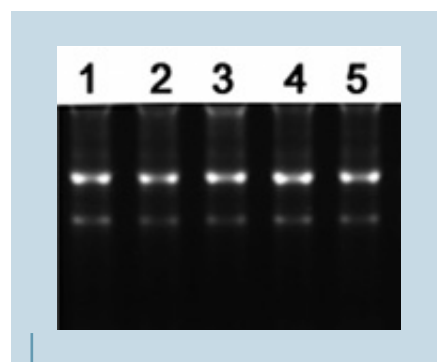
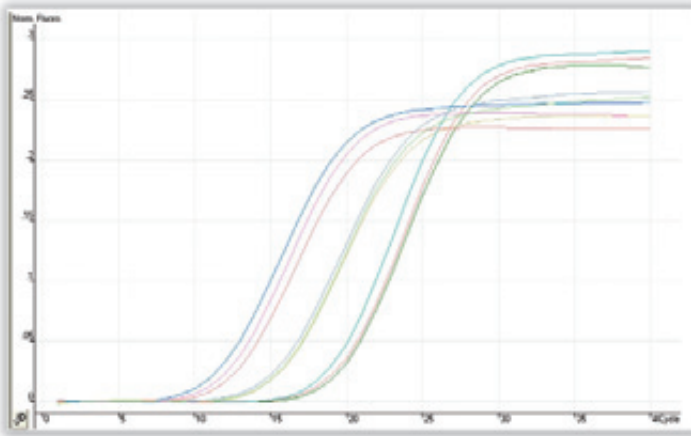
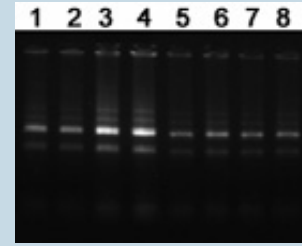


Figure 1. 1% agarose gel analysis of total RNA isolated with E.Z.N.A.™ Total RNA Kit I from Cos-7 cells (1), Hela cells (2), LMH cells (3), Mouse Liver (4) and Mouse Speed (5). 5% of purified RNA were loaded on gel.



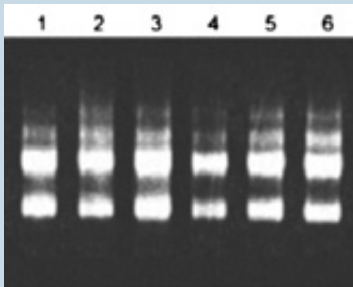
**Figure 2.** RNA was purified from HeLa cells, Cos-7 cells and 293 cell using the E.Z.N.A.™ Total RNA Kit I (treated with DNase I). After reverse transcription, real-time, quantitative PCR® was carried out on the Rotor-Gene 3000 and PCR® specific for  $\beta$ -actin.



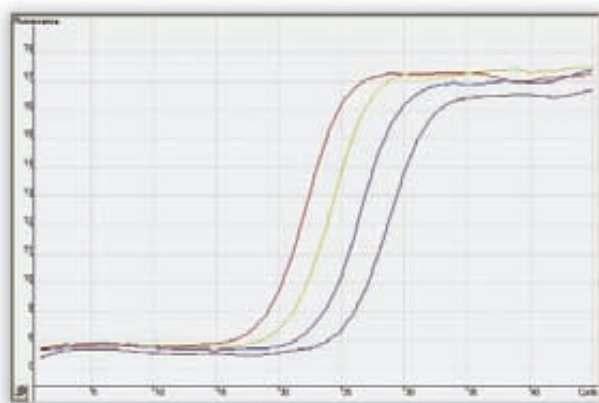
**Figure 3.** 1% agarose gel analysis of total RNA isolated from plant with two protocols from E.Z.N.A.™ Plant RNA Kit: Standard protocol: 1: Paddle leaves, 2: lichi leaves, 3: Tomato leaves, 4: Maize Leaves: Optional protocol for difficult samples: 5: Paddle seeds, 6: Maize seeds, 7: Potato leaves, 8: pumpkin leaves.

## E.Z.N.A.™ Total RNA Kit II (R6934)

The E.Z.N.A. Total RNA Kit II takes the advantage of one step RNA isolation technology and silica-membrane technology and combines these two systems together. Although this kit is mainly designed for fatty tissues, it can be used for almost all types of biological samples. With two extraction steps, this kit can significantly reduce the level of genomic DNA contamination (Figure 4) and increase the purity of RNA. Figure 5 demonstrates result from real-time RT-PCR®.



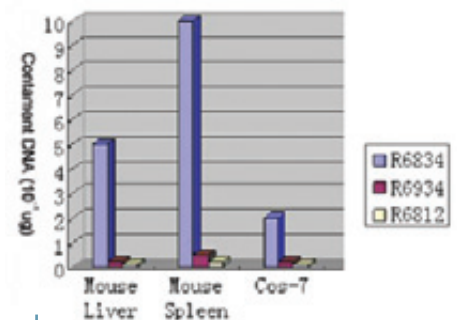
**Figure 4.** 1% agarose gel analysis of RNA purified with E.Z.N.A.™ Total RNA Kit II from pig heart (1), liver (2), kidney (3), fat (4), spleen (5) and brain (6). 1% of purified RNA was loaded on gel.



**Figure 5.** RNA was purified from pig fatty tissue using the E.Z.N.A.™ Total RNA Kit II. After reverse transcription using M-MLV Reverse Transcriptase, real-time, quantitative PCR® was carried out on the Rotor-Gene 3000 and primers specific for  $\beta$ -actin.

## E.Z.N.A.™ HP Total RNA Kit (R6812)

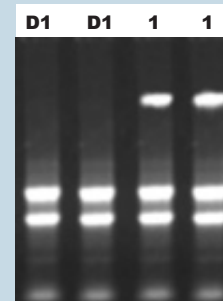
The E.Z.N.A.™ HP Total RNA Kit is designed for isolating RNA from small amounts of cells and tissues. By using a specially designed genomic DNA removal spin column, this kit effectively removes genomic DNA. To show the efficacy of this kit, Mouse Liver, Mouse Spleen and Cos-7 cells were used isolate RNA with three kits: E.Z.N.A.™ Total RNA Kit I, E.Z.N.A.™ Total RNA Kit II and E.Z.N.A.™ HP Total RNA Kit. After Purification, the RNA were treated with RNase A (No DNase I detected) and then extracted by phenol, precipitated by isopropanol, and any DNA re-dissolved by Buffer TE. DNA contamination level using different kits is shown in Figure 6.



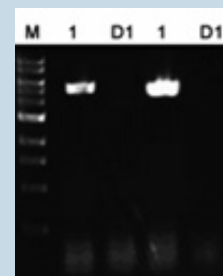
**Figure 6.** RNA was isolated from different samples with three kits. And the DNA contaminants are showed as figure.

## On-Membrane DNase I digestion (E1091)

Omega Bio-tek has developed an on-membrane DNase I protocol that can be use in all E.Z.N.A.™ RNA Kits to remove the genomic DNA during RNA isolation process. To show the efficiency of on-membrane DNase I digestion, the RNA and DNA were bound to a column, then treated with DNase I (E1091) as per kit instructions, the results were showed in Figure 7 and Figure 8.



**Figure 7.** Effect of On-Membrane DNase I digestion treatment: Total RNA isolated with E.Z.N.A.™ Total RNA Kit I from Cos-7 cells. Then the column is treated with DNase I (D1) or without treated (1). RNA eluted with DEPC-Treated water. 10% of eluted RNA was analysed by 1% agarose gel.



**Figure 8.** RNA treated with (D1) or without (1) DNase I on membrane, then PCR® amplified  $\beta$ -actin.

## High quality RNA isolation using E.Z.N.A.™ RNA system

(continued)

Samples	Product	Size	Cat. No.
Soft animal tissue: 30 mg	Total RNA Kit I	50	OMEGR6834-01
Culture cells: 1 x 10 <sup>7</sup> cells		200	OMEGR6834-02
Soft animal tissue: 100 mg	Total RNA Midi Kit	10	OMEGR6664-01
Culture cells: 1 x 10 <sup>8</sup> cells		25	OMEGR6664-02
Soft animal tissue: 1g	Total RNA Maxi Kit	5	OMEGR6693-01
Culture cells: 5 x 10 <sup>8</sup> cells		20	OMEGR6693-02
100 mg tissue or 1 x 10 <sup>7</sup> culture cells	Total RNA Kit II	50	OMEGR6934-01
		200	OMEGR6934-02
Fibrous or fixed tissue: 30 mg	Tissue RNA Kit	50	OMEGR6688-01
		200	OMEGR6688-02
Tissue: <10 mg, Cells: < 1 x 10 <sup>6</sup> cells	MicroElute RNA Kit	50	OMEGR6831-01
		200	OMEGR6831-02
1 ml blood	Blood RNA Kit	50	OMEGR6814-01
		200	OMEGR6814-02
10 ml blood	Blood RNA Midi Kit	10	OMEGR6615-01
		25	OMEGR6615-02
50 ml blood	Blood RNA Maxi Kit	5	OMEGR6616-01
		20	OMEGR6616-02
100 mg plant	Plant RNA Kit	50	OMEGR6827-01
		200	OMEGR6827-02
500 mg plant	Plant RNA Midi Kit	10	OMEGR6628-01
		25	OMEGR6628-02
5 g plant	Plant RNA Maxi Kit	5	OMEGR6629-01
		20	OMEGR6629-02
100 mg fungal	Fungal RNA Kit	50	OMEGR6840-01
		200	OMEGR6840-02
1-3 ml log phase bacterial	Bacterial RNA Kit	50	OMEGR6950-01
		200	OMEGR6950-02
1-3 ml log phase yeast	Yeast RNA Kit	50	OMEGR6870-01
		200	OMEGR6870-02
Serum and cell free body fluids	Viral RNA Kit	50	OMEGR6874-01
		200	OMEGR6874-02
Cell and tissues	DNA/RNA Kit	50	OMEGR6731-01
		200	OMEGR6731-02
Soft animal tissue: 30 mg	E-Z 96 Total RNA Kit	4 x 96	OMEGR1034-01
Culture cells: 1 x 10 <sup>7</sup> cells		12 x 96	OMEGR1034-02
Fibrous or fixed tissue: 30 mg	E-Z 96 Tissue RNA Kit	2 x 96	OMEGR1088-01
		8 x 96	OMEGR1088-02
Serum and cell free body fluids	E-Z 96 Viral RNA Kit	4 x 96	OMEGR1074-01

## Conclusion and discussion

The E.Z.N.A.™ RNA range includes specialised kits for different types of starting materials include all types of animal tissues, bacteria, plant, fungi, yeast and molluscs. Different kit formats are available handling small and large sample sizes and for manual and automated processing of up to 96 or 192 samples in parallel. A previous study showed that no existing RNA isolation method can completely avoid the genomic DNA contamination (Vongsavanh, 2002). Omega Bio-Tek has developed two methods to significantly reduce the genomic DNA contamination. The E.Z.N.A.™ RNA purification kits, which are based on silica-membranes and Mag-Bind™ RNA purification kits provide a complete solution for consistent yield of high quality RNA.

Omega Bio-tek has developed a full range of RNA isolation kits to provide reliable RNA purification procedures based on the unique properties of biological samples. For example, when purifying RNA from animal tissue, some samples such as liver can be easily lysed with lysis buffer, while other samples such as brain tissue or muscle tissues require special treatments for lysis. Plant tissues contain different levels of phenolic compounds and/or polysaccharides. The E.Z.N.A.™ RNA Purification kits, which are all based on silica-membrane technology except Mag-Bind™ RNA purification kits, provide a completely solution for consistent yield of highly pure RNA. Visit [www.omegabiotek.com](http://www.omegabiotek.com) to find out more about new solutions for RNA purification.



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