A Performance Comparsion Study: Omega Bio-tek's E.Z.N.A.® HP Total RNA Kit (R6812) vs. Qiagen's RNeasy Plus Mini Kit (74134)

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Introduction

Extraction of sufficient amounts of high quality RNA is a crucial step in generating the most sensitive and relevant results downstream. Among a variety of commercially available extraction kits on the market, we pit the performance of our E.Z.N.A.® HP Total RNA Kit (R6812) against the popular RNeasy Plus Mini Kit (74134). Both the kits are capable of efficiently purifying up to 100 µg of total RNA (> 200 nt) from a single extraction along with an effective on-column gDNA removal. RNA was isolated from tissue samples as well as cultured cells and the kit performance was evaluated based on 3 parameters -- quantity, quality, and integrity. The results of the comparion study are reported here.

Materials & Methods

Total RNA was purified from either 12.5 mg of mouse kidney or 2.2x10^6 human embryonic kidney cells (HEK293) using Omega Bio-tek's HP Total RNA Kit and Qiagen's RNeasy Plus Mini Kit. Experiments were performed on replicates of 4 each. The extraction workflow of the kits is depicted in Figure 1. The number of steps involved are comparable and the entire procedure took about 25 minutes with both of the kits.

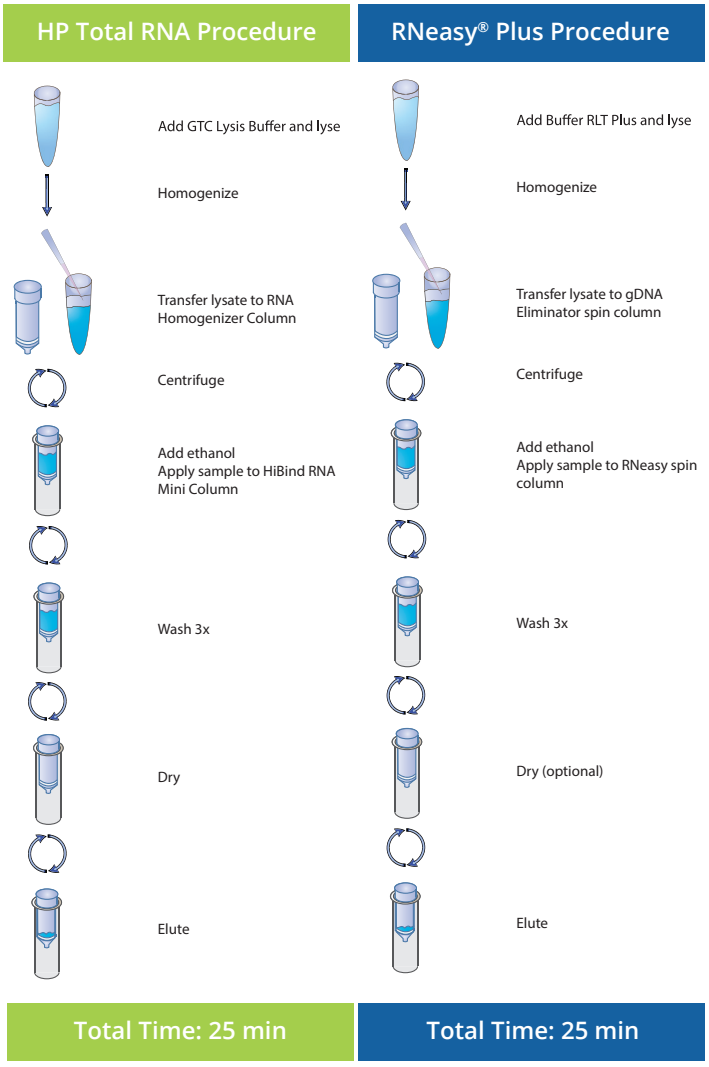

Figure 1. RNA extraction workflow comparison.

Quantity & Quality Assessment

The RNA yield was quantified using Thermo Scientific's NanoDrop™ 2000c. DNA co-eluted with RNA was quantified using QuantiFluor® dsDNA system. The QuantiFluor® dsDNA system selectively binds to double-stranded DNA (dsDNA) in solution. It shows minimal binding to single-stranded DNA (ssDNA) and RNA, allowing specific quantitation of dsDNA. The percent gDNA contamination was estimated as the ratio of DNA quantified using QuantiFluor® dsDNA system to the total RNA estimated using Thermo Scientific's NanoDrop™ 2000c.

Integrity Assessment

RNA isolated was analyzed on Agilent's TapeStation® 2200, which measures the RNA integrity, displayed as the RNA Integrity Number (RIN). RIN ranges from 0 to 10, with 10 indicating maximum RNA integrity. Lately, RIN is being considered a de facto standard for RNA integrity as it takes into account the entire electrophoretic trace, not just the ratio of 28S and 18S rRNAs.

Results

Figure 2 shows the RNA yields obtained using the Omega and Qiagen kits with tissue samples and cultured cell samples. RNA yields were 18.36 µg and 28.17 µg from the mouse kidney, 17.32 µg and 19.96 µg from the HEK293 cells using Qiagen and Omega Bio-tek kits respectively. One-way ANOVA followed by *Tukey's post-hoc* analysis indicates that the RNA yield extracted using Omega Bio-tek's kit significantly better than that of Qiagen's ($p < 0.01$) for both the sample types tested.

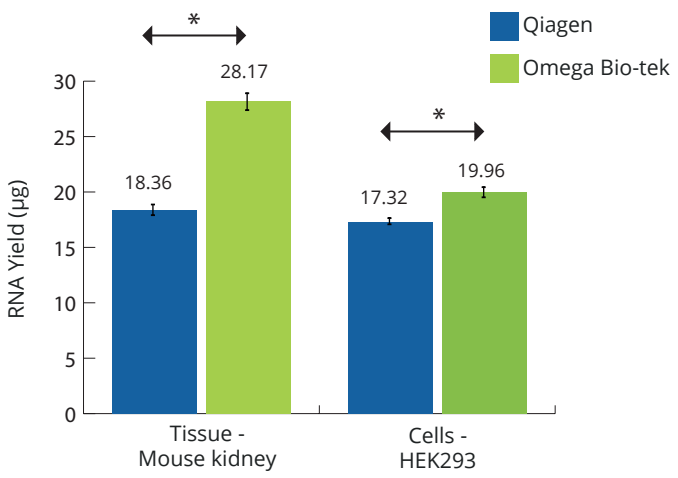

Figure 2. RNA yield comparison. *p < 0.01.

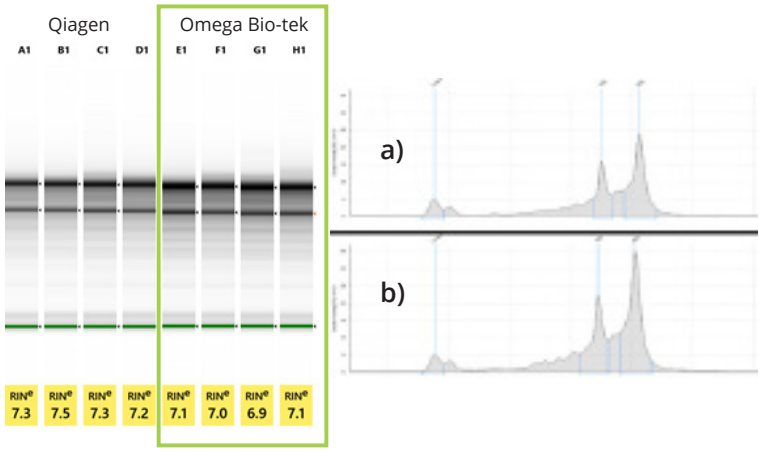

Figure 3. RNA integrity analysis - tissue sample. a) Representative electropherogram - Qiagen; b) representative electropherogram - Omega Bio-tek.

Genomic DNA contamination in the isolated DNA is shown in Table 1. With the RNeasy Plus Kit, DNA co-eluted was found to be 4.52% of total RNA for both the sample types tested. And it was 4.79% for HP Total RNA Kit for the same. The findings suggest that the RNA isolated from both the kits was of high quality with appreciable gDNA removal.

Table 1. Percent gDNA contamination of the isolated RNA.

Company	Sample Type	% gDNA Contamination
Qiagen	Tissue - Mouse kidney	4.52 ± 0.01
Omega Bio-tek		4.79 ± 0.002
Qiagen	Cells - HEK293	4.52 ± 0.002
Omega Bio-tek		4.79 ± 0.001

The purified RNA was also analyzed on the Agilent TapeStation® 2200 to provide information on its integrity. The average RIN numbers were 7.325 and 7.025 for the tissue samples (mouse kidney) using Qiagen and Omega Bio-tek kits, respectively. For cultured cells, the average RIN number was 10 irrespective of the kit used. Gel images, along with RIN numbers and representative electropherograms for the sample types used, are shown in Figures 3 and 4. The individual RIN scores suggest that isolated RNA is of high quality and acceptable for various downstream applications.

Discussion

The RNA yields were significantly higher from the tissue sample as well as the cultured cells isolated using the Omega Bio-tek kit than with the Qiagen kit. The cost with the Qiagen kit is $6.64 per preparation whereas it is $3.90 with Omega Bio-tek. For an end user, that is a significant 41% savings with Omega Bio-tek for comparable or better results.

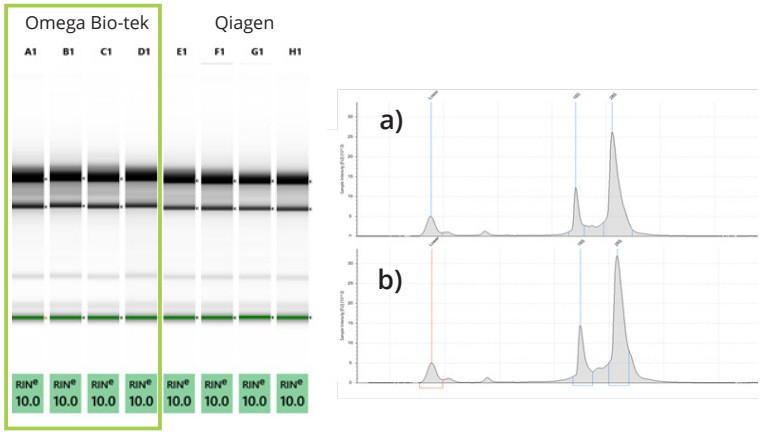

Figure 4. RNA integrity analysis - cultured cells. a) Representative electropherogram - Qiagen; b) representative electropherogram - Omega Bio-tek.

Product Information

Description	Product No.	Preps
E.Z.N.A.® HP Total RNA Kit	R6812-00	5
	R6812-01	50
	R6812-02	200

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