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Life Sciences

Total RNA Isolation and Purification from Cultured Eukaryotic Cells

RNAdvance Cell v2

The RNAdvance Cell v2 kit is an RNA isolation reagent kit buit on (SPRI) paramagnetic bead-based technology. It enables the purification of high quality RNA from cell lines. The extraction can be run manually in a 2 mL tube format or a 96-well format, or automated in a 96-well format on variety of Beckman Coulter Biomek liquid handling workstations. Total RNA extracted using RNAdvance Cell v2 kit is free of detectable gDNA and other PCR inhibitors and suitable for NGS,microarrays, and qRT-PCR applications.

- Efficient removal of genomic DNA and other contaminants
- Extraction and purification of high quality total RNA from cultured cells supports extraction of a large numbers of samples for gene expression
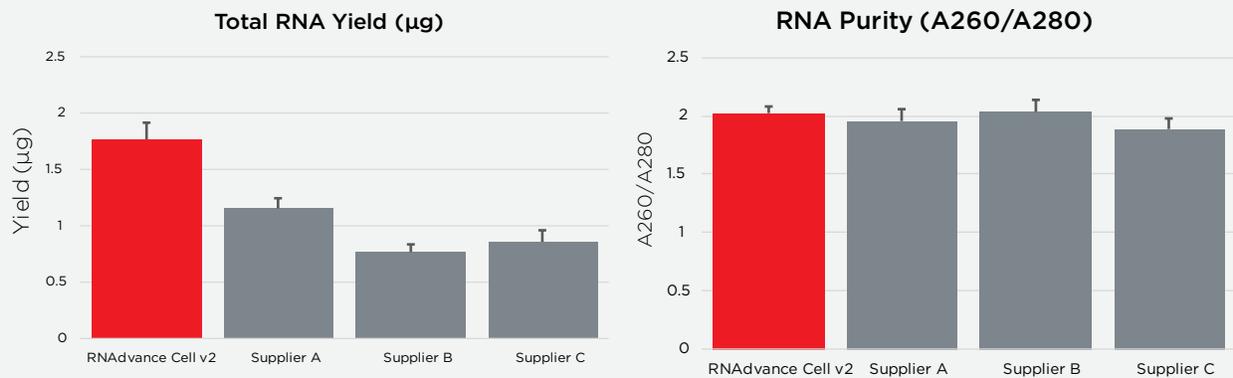
High recovery of RNA by RNAdvance Cell v2

Figure 1. RNA was extracted from MRC-5 cells using RNAdvance Cell V2 and other suppliers' kits. (Left) Samples were quantified using the Nanodrop (Thermo Fisher Scientific). Higher amounts of RNA were recovered using the RNAdvance Cell V2 kit over three of the other Suppliers' kits. (Right) Samples were accessed for purity using the Nanodrop (Thermo Fisher Scientific). For MRC-5, RNAdvance Cell V2 purified RNA with A260/280 ratios within satisfactory ratios

Efficient removal or genomic DNA and PCR inhibitors allows for quality data and results in qRT-PCR applications
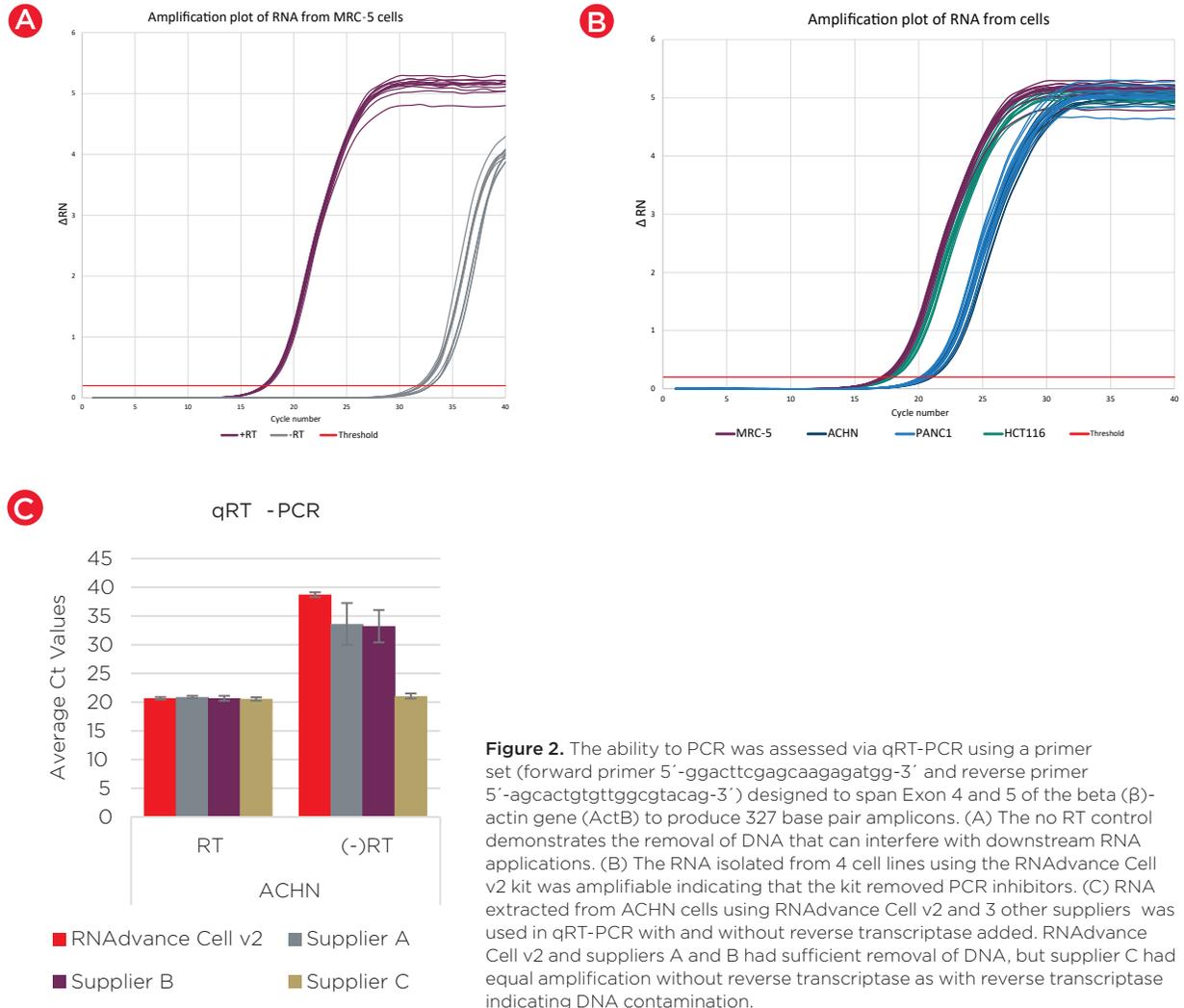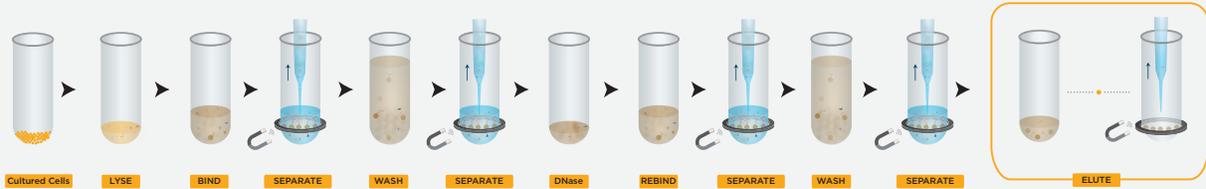
A Amplification plot of RNA from MRC-5 cells

+RT · -RT · Threshold

B Amplification plot of RNA from cells

MRC-5 · ACHN · PANC1 · HCT116 · Threshold

C qRT - PCR

ACHN

RNAdvance Cell v2 · Supplier A
Supplier B · Supplier C

Figure 2. The ability to PCR was assessed via qRT-PCR using a primer set (forward primer 5´-ggacttcgagcaagagatgg-3´ and reverse primer 5´-agcactgtgttggcgtacag-3´) designed to span Exon 4 and 5 of the beta (β)-actin gene (ActB) to produce 327 base pair amplicons. (A) The no RT control demonstrates the removal of DNA that can interfere with downstream RNA applications. (B) The RNA isolated from 4 cell lines using the RNAdvance Cell v2 kit was amplifiable indicating that the kit removed PCR inhibitors. (C) RNA extracted from ACHN cells using RNAdvance Cell v2 and 3 other suppliers was used in qRT-PCR with and without reverse transcriptase added. RNAdvance Cell v2 and suppliers A and B had sufficient removal of DNA, but supplier C had equal amplification without reverse transcriptase as with reverse transcriptase indicating DNA contamination.

Visual Workflow

Cultured Cells · LYSE · BIND · SEPARATE · WASH · SEPARATE · DNase · REBIND · SEPARATE · WASH · SEPARATE · ELUTE

1. Lyse cells in Lysis Buffer and Proteinase K
2. Bind RNA to magnetic beads
3. Separate magnetic beads from contaminants
4. Wash magnetic beads with Wash Buffer and 70% ethanol to remove contaminants
5. Treat samples with DNase I
6. Rebind RNA to magnetic beads with Wash Buffer
7. Wash magnetic beads with 70% ethanol to remove contaminants
8. Elute RNA from magnetic beads
9. Transfer to a new plate for storage

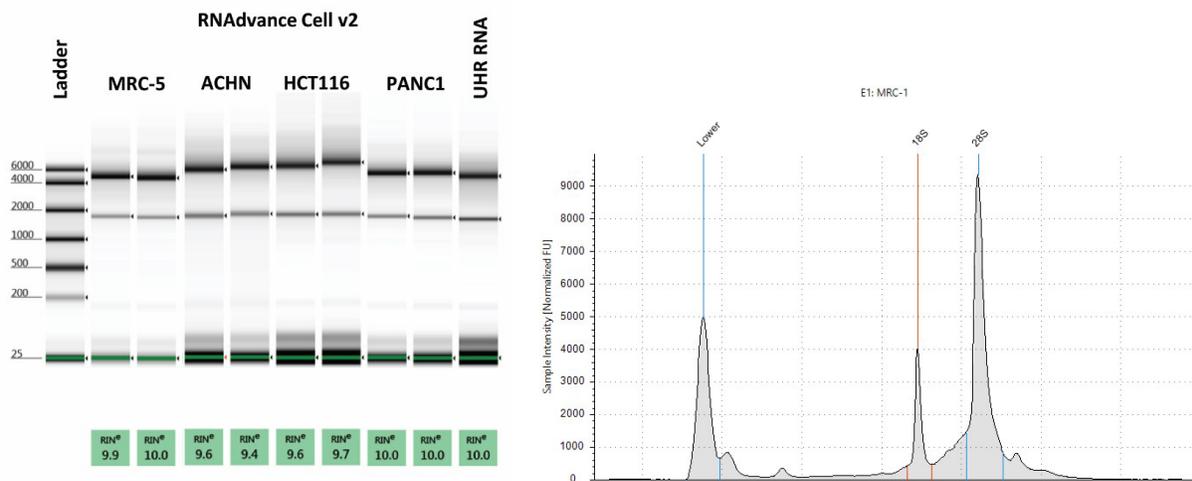# Isolate high Quality RNA with RIN scores averaging 9.8 from a variety of cell lines

Figure 3. RNAdvance Cell v2 isolates high quality RNA. Total RNA extracted from MRC-5, ACHN, HCT116, and PANC1 cells and universal human reference RNA (Agilent) were run on the Agilent RNA ScreenTape to assess quality. (Left) RIN values from all samples isolated with RNAdvance Cell v2 were all ≥ 9.4 indicating that high quality and intact RNAs were recovered. (Right) A sample trace of RNA isolated from MRC-5 cells corresponding to the first lane of the gel is shown; the 18s and 28s rRNA peaks are prominent.

RNA can be extracted from a variety of Cell Lines

Cell Type	Yield (µg)	$A_{260/280}$
MRC-5	1.8	2.02
ACHN	0.5	1.98
HCT116	0.3	2.00
PANC1	1.0	1.93

Table 1. RNA was extracted from 5x104 MRC-5, ACHN, HCT116 and PANC1 cells using RNAdvance Cell v2. Samples were quantified and accessed for purity using the NanoDrop (Thermo Fisher Scientific). RNA yield is cell line dependent, but RNA quality is consistent between all cell lines and is within satisfactory A260/280 ratios.

Users can extract RNA from samples in less time with less pipette actions compared to users of column based kits
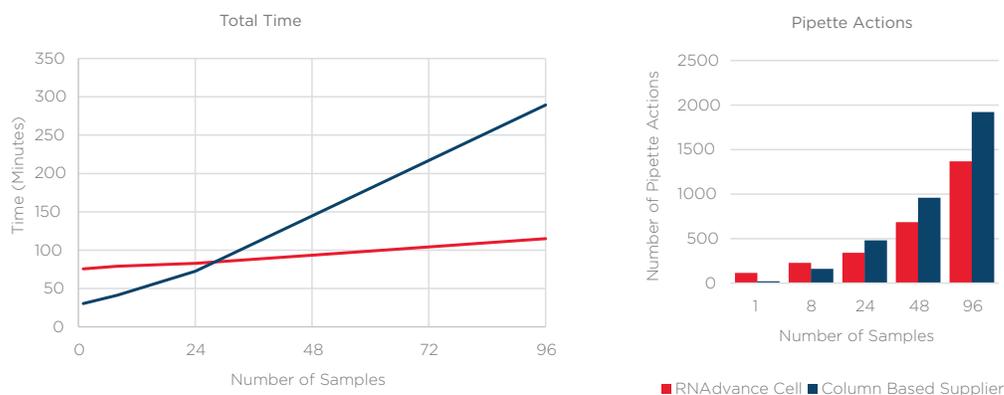

Figure 4. (Left) Represents total time to extract RNA for 1 to 96 samples using RNAdvance Cell v2 or a column based supplier. At 30 samples total time to extract RNA from cells is faster using RNAdvance Cell v2. (Right) The total number of pipette actions, which include dispensing in a sample, mixing a sample, and discarding tips, required for 1, 8, 24, 48, and 96 samples. With the ability to use a multichannel pipette there is significantly less pipette actions that need to take place than with column based suppliers.

# For use in manual or automated methods based on batch size or overall throughput

			RNAdvance Cell v2	
			Manual	Automated
Batch Size	8	Hands-on Time	1.00	0.25
		Total Time	2.70	2.43
	24	Hands-on Time	1.50	0.25
		Total Time	3.20	2.50
	48	Hands-on Time	NR	0.50
		Total Time	NR	2.86
	96	Hands-on Time	NR	0.50
		Total Time	NR	3.08

Table 2. Estimated hands-on time and total time in hours, required to perform 8, 24, 48 and 96 RNA extractions The methods can be performed either manually or automated. Times represented in this table are based on a Biomek i7 Hybrid. Difference in time between manual and automation is indicated. NR=Not Recommended.

Product infoRMATION

PART NO	NAME	PREPS
A47942	RNAdvance Cell v2 Kit	100
A47943	RNAdvance Cell v2 Kit	960

For more information, please contact:
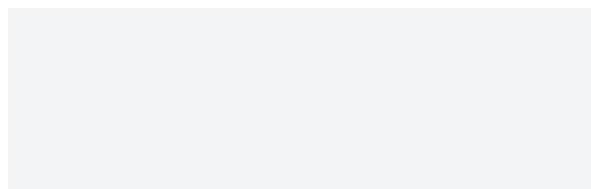
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