

Applied Biosystems® Arcturus® Paradise® PLUS Whole Transcript Reverse Transcription Reagent System

Benefits

- Simple workflow—minimal sample handling reduces chance of contamination
- Efficient RNA extraction and isolation—helps save time and minimizes sample loss
- Optimized RT reactions—generate cDNA representing the entire length of the transcript
- Enables accurate transcript analysis—results represent high-, medium-, and low-abundance expressing genes



Figure 1. The Applied Biosystems® Arcturus® Paradise® PLUS Whole Transcript Reverse Transcription Reagent System.

Introduction

The Applied Biosystems® Arcturus® Paradise® PLUS Whole Transcript Reverse Transcription (WT-RT) Reagent System from Life Technologies provides an efficient and reliable solution for purifying RNA from archived formalin-fixed, paraffin-embedded (FFPE) tissue samples, followed by reverse transcription of the whole transcript for use in real-time PCR reactions (Figure 1).

FFPE samples introduce unique challenges for gene expression profiling and gene expression quantitation, including chemical modification and fragmentation of RNA molecules. Archiving of FFPE samples adds to these challenges through increased RNA degradation over time.

The Paradise® PLUS WT-RT Reagent System was specifically developed and specially optimized to provide high-quality RNA and robust reverse transcription (RT) from highly fragmented RNA obtained from archived FFPE samples over 20 years old (Table 2).

Optimized RNA Extraction and Isolation from Archived FFPE Tissues

Table 1 shows a comparison of total RNA yields using the Paradise® PLUS WT-RT Reagent System and a similar commercially available product. The average yield of the three replicate isolations illustrates the abundance of total RNA made available using the Paradise® PLUS WT-RT Reagent System. The Paradise® PLUS WT-RT Reagent System produces abundant total RNA for reverse transcription and subsequent quantitative real-time PCR analysis, up to three times more total RNA than obtained when a similar commercially available product was used for the same sample.

Table 1. RNA yield comparison.

Sample ID	Sample Age	RNA Isolation Protocol	A_{260}/A_{280}	Average Yield (ng)
9055	1 year	WT-RT	1.94	7,592.8
		Vendor A	2.05	2,086.3
8090	10 years	WT-RT	1.95	784.4
		Vendor A	2.45	247.4

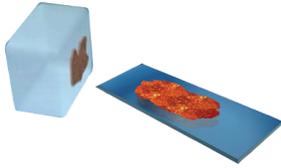
Superior Amplification Enabled Over a Wide Range of Targets

The sensitivity of the Paradise® PLUS WT-RT Reagent System was compared to similar reagent products available on the market that generate cDNA for use in qPCR reactions. As Figure 3 demonstrates, improved C_t values were seen for all high-, medium-, and low-expressing genes when the Paradise® PLUS product was used. Exon-spanning primers at varying distances from the 3' end of the transcript (500 bp to 5 kb) were used to show increased sensitivity and improved transcription success compared to other commercially available products. The Paradise® PLUS WT-RT Reagent System successfully amplified all genes, demonstrating that the reagents provided in the kit are optimized to work together, which helps save you time and effort while producing high-quality results across the entire transcript.

Table 2. Quantitative Real-Time RT-PCR Analysis of High-, Medium-, and Low-Expressing Genes for Archived FFPE Tumor Tissue Samples.

Sample ID	Tissue Type	Tissue Age (Years)	Housekeeping	High Expressed	Medium Expressed	Low Expressed
			C _t	C _t	C _t	C _t
5026	Breast	22	23.11	32.42	35.47	31.58
5028	Breast	20	27.62	37.37	36.96	35.94
5031	Breast	18	22.83	30.61	37.73	29.53
5113	Breast	10	23.37	33.65	38.48	31.43
5111	Breast	8	21.56	31.07	31.65	28.32
53635	Brain	<1	23.25	31.01	40.00	28.09
53808	Lymphoid tissue	<1	24.89	38.55	40.00	33.35
53720	Ovary, right	<1	24.11	37.39	40.00	33.24
53855	Endometrium	<1	25.12	34.76	40.00	32.96
53825	Prostate	<1	26.60	33.55	30.81	35.26
53958	Bone	<1	23.49	37.28	34.42	35.14
53780	Testis	<1	20.88	32.08	34.78	32.48
53853	Pancreas	<1	24.53	34.43	36.72	34.35
Universal RNA	—	—	24.81	35.43	34.47	32.67
Universal RNA	—	—	23.88	33.56	35.29	32.25

Note: "RT negative" reactions were run in parallel for representative genes for each sample. Housekeeping gene = beta-actin, high expressed gene = CHDH, medium expressed gene = HoxB13, low expressed gene = IL.



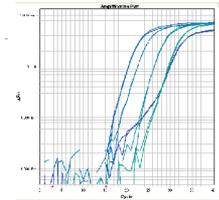
(1) Cut a 7 micron section (25–150 mm²) from a paraffin block. Remove sample from paraffin while keeping RNA intact.



(2) Recover high-quality total RNA using optimized extraction and isolation reagents.



(3) Synthesize quality cDNA representing entire transcript length with qPCR application.



(4) Validate biomarkers previously identified during gene expression profiling.

Figure 2. Whole Transcript Reverse Transcription Process.

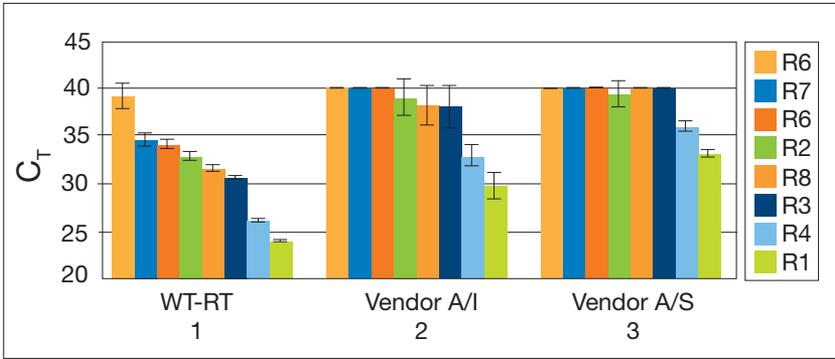


Figure 3. Increased Sensitivity Comparison of Paradise® PLUS WT-RT Kit and Similar Commercially Available Products. (1) Total RNA from FFPE breast cancer tissue was extracted, isolated, and reverse transcribed using the Paradise® PLUS WT-RT Reagent System. (2, 3) Alternatively, RNA from the same FFPE tissue sample was extracted and isolated using a kit from Vendor A, then reverse transcribed using a kit from (2) Vendor I or (3) Vendor S. Total RNA input for all RT reactions was consistent at 100 ng. The resulting cDNA from all vendors was used for qPCR.

ORDERING INFORMATION

Description	Size	Part Number
Paradise® PLUS WT-RT Reagent System		
Paradise® PLUS Whole Transcript RT Reagent System	12 reactions	KIT0315
Paradise® PLUS Whole Transcript RT Reagent System	48 reactions	KIT0305
Additional FFPE Reagent Products		
The Paradise® PLUS Reagent System includes reagents for sample preparation, tissue staining, RNA extraction, isolation, RNA amplification, and microarray labeling.		
Paradise® PLUS WT-RT Reagent System with Biotin Labeling	12 two-round reactions	KIT0312B
Paradise® PLUS Reagent System with Cy®3 Labeling	12 two-round reactions	KIT0312C
Paradise® PLUS Reagent System with Cy®5 Labeling	12 two-round reactions	KIT0312D
Paradise® PLUS Reagent System with Amino Alkyl Labeling	12 two-round reactions	KIT0314
Paradise® PLUS Reagent System for Quantitative Real-Time PCR	12 two-round reactions	KIT0310
Paradise® PLUS Reagent System for Quality Assessment of FFPE Block (QC Kit)	12 two-round reactions	KIT0313

To learn more about the Arcturus® Paradise® PLUS Whole Transcript RT Reagent System from Applied Biosystems, contact your local sales representative, or visit us at www.appliedbiosystems.com/arcturus.

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