Magen FFPE RNA Kit Test Report

Performance validation for HiPure FFPE RNA Kit (R4143), HiPure FFPE RNA Plus Kit (R4144), and MagPure FFPE RNA Kit (IVD3022)

Consolidated performance validation report

1. Report Scope

This report summarizes performance data for Magen FFPE RNA extraction workflows. It covers the column-based HiPure FFPE RNA Kit (R4143), the DNase I-containing HiPure FFPE RNA Plus Kit (R4144), and the magnetic bead-based MagPure FFPE RNA Kit (IVD3022).

R4143 and R4144 use the same core column-based FFPE RNA workflow. The key difference is that R4144 includes DNase I and DNase Booster Buffer for stronger removal of residual genomic DNA. IVD3022 uses magnetic particles and includes DNase I digestion, supporting manual, liquid-station, and KingFisher-type magnetic workflows.

Cat.	Product	Format	DNase I	Use
R4143	HiPure FFPE RNA	Column	No	Standard FFPE RNA
R4144	HiPure FFPE RNA Plus	Column	Yes	gDNA control
IVD3022	MagPure FFPE RNA	Magnetic	Yes	Magnetic / automation

2. FFPE Tissue RNA Extraction Performance - R4143 / R4144 Column Workflow

FFPE tissue RNA extraction performance was evaluated using one-year paraffin-embedded tissue samples, including grass carp liver, grass carp muscle, chicken liver, and chicken heart. A 9 mg FFPE tissue input was processed using the HiPure FFPE RNA column workflow. Purified RNA was measured by Nanodrop and analyzed by 2% agarose gel electrophoresis.

Sample	Conc. (ng/μL)	A260/280	A260/230	Yield (μg)
Fish liver	372.1	1.88	1.13	14.8
Fish liver	574.7	1.78	0.94	22.9
Fish muscle	39.1	1.69	0.36	1.5
Fish muscle	44.7	1.75	0.39	1.7
Chicken liver	532.3	1.96	1.38	21.2
Chicken liver	627.7	1.92	1.21	25.1
Chicken heart	70.1	1.84	0.58	2.804
Chicken heart	71.8	1.84	0.57	2.8

From 9 mg FFPE fish liver input, the column workflow produced RNA yields of 14.8-22.9 μg. From 9 mg FFPE chicken liver input, RNA yields were 21.2-25.1 μg. Lower-yielding FFPE tissue types, including fish muscle and chicken heart, produced 1.5-1.7 μg and approximately 2.8 μg RNA, respectively, under the tested conditions.

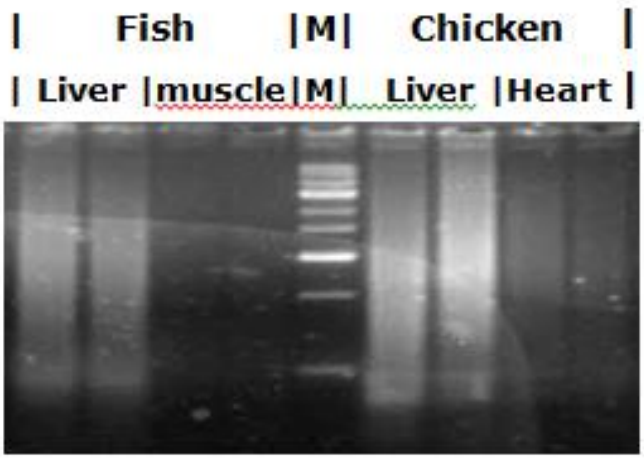


Figure 1. Electrophoresis analysis of RNA extracted from FFPE fish liver, fish muscle, chicken liver, and chicken heart samples.

3. DNA Contamination Assessment

Purified FFPE chicken liver RNA from the column workflow was further treated with DNase I and analyzed by electrophoresis. The electrophoresis pattern showed no obvious change before and after DNase I digestion, indicating that the RNA obtained with the standard HiPure FFPE RNA Kit contained little visible DNA contamination under the tested conditions.

This result is related to the nature of FFPE samples. DNA can remain cross-linked with histones in paraffin-embedded tissue, and during short digestion, DNA may not fully dissociate into the lysate. Therefore, the standard R4143 workflow can show low visible DNA carryover in the tested FFPE RNA extraction conditions.

HiPure FFPE RNA Plus Kit (R4144) is designed for stronger genomic DNA control. It uses a DNase digestion process in which DNase Booster Buffer is added directly into the digestion solution. The booster buffer helps shield DNase I from the inhibitory effects of SDS and protease, allowing DNase I to remain active in the digestion mixture and digest residual DNA. This design supports recovery of RNA with reduced genomic DNA background for downstream applications sensitive to DNA contamination.

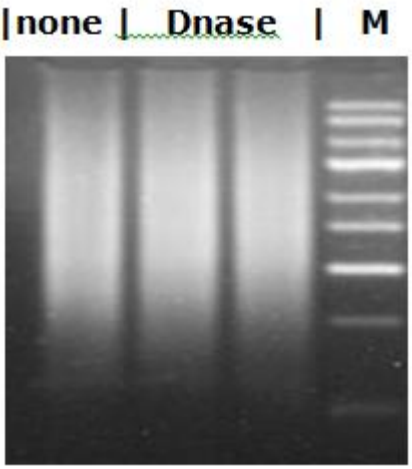


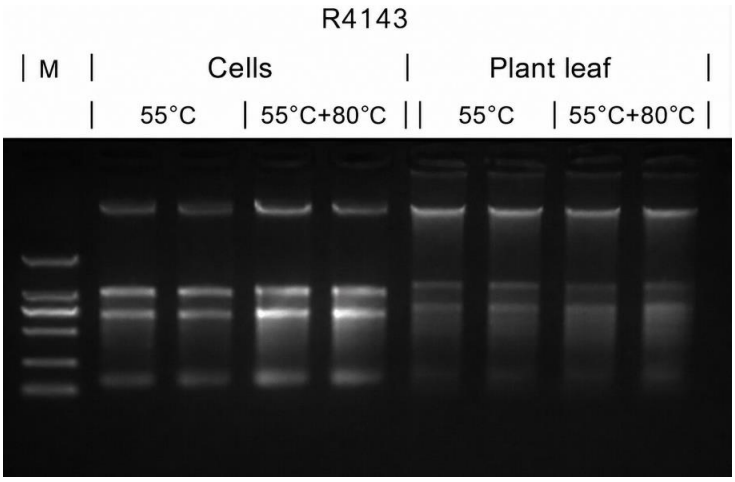
Figure 2. Electrophoresis comparison of FFPE chicken liver RNA before and after DNase I digestion.

4. Heat Treatment and RNA Integrity

The R4143 workflow includes an 80°C heat-treatment step to help reverse formaldehyde-related nucleic acid modification. To evaluate whether this step affects RNA integrity, model samples were processed under two digestion conditions: 55°C digestion and 55°C digestion followed by 80°C treatment. The samples included 200 µL cell input and 6 mg plant leaf input.

Sample	Input	Condition	Conc. (ng/µL)	Yield (µg)	A260/280	A260/230
Cells	200 µL	55°C	260.889	13.044	2.093	1.446
Cells	200 µL	55°C	229.140	11.457	2.007	0.995
Cells	200 µL	55°C+80°C	417.539	20.877	2.124	1.162
Cells	200 µL	55°C+80°C	331.498	16.575	2.140	1.709
Plant leaf	6 mg	55°C	101.452	5.073	1.944	1.145
Plant leaf	6 mg	55°C	128.288	6.414	1.952	1.419
Plant leaf	6 mg	55°C+80°C	92.640	4.632	1.955	0.291
Plant leaf	6 mg	55°C+80°C	98.409	4.920	1.990	0.199

Electrophoresis analysis showed that RNA extracted from cells and plant leaves did not show visible degradation after the 80°C treatment. Eluted RNA stored overnight at 4°C also maintained clear band patterns. These data support the use of the heat-treatment step as workflow-supporting evidence for FFPE RNA processing.



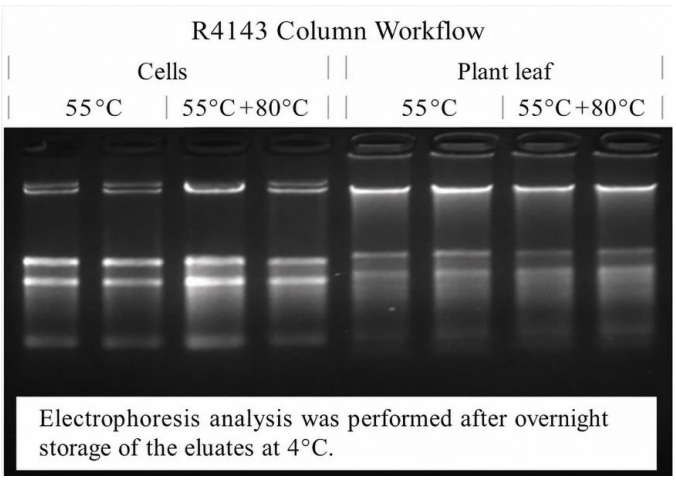


Figure 3. Electrophoresis comparison of RNA extracted after 55°C digestion and 55°C + 80°C treatment.

Figure 4. Electrophoresis analysis of eluted RNA after overnight storage at 4°C.

5. RT-PCR Compatibility

RT-PCR compatibility was evaluated by adding Newcastle disease virus RNA into the lysis buffer system, followed by extraction and fluorescent RT-PCR analysis. The final PCR reaction used 10 μL of recovered sample. R4143 extracts showed Ct values close to the standard viral reference reagent under the tested conditions, indicating no obvious PCR inhibition from the extraction workflow.

Test Condition	Replicate / Note	Ct
Viral reference	Virus	21.76
Viral reference	Virus	22.16
Viral reference	Water control	22.88
Viral reference	Water control	22.83
R4143	Reference batch	20.11
R4143	Reference batch	20.42
R4143	QC batch	20.60
R4143	QC batch	20.10

The RT-PCR amplification curves were consistent with the Ct results. No obvious inhibition was observed in R4143 extracts, and no meaningful batch-to-batch difference was observed under the tested conditions.

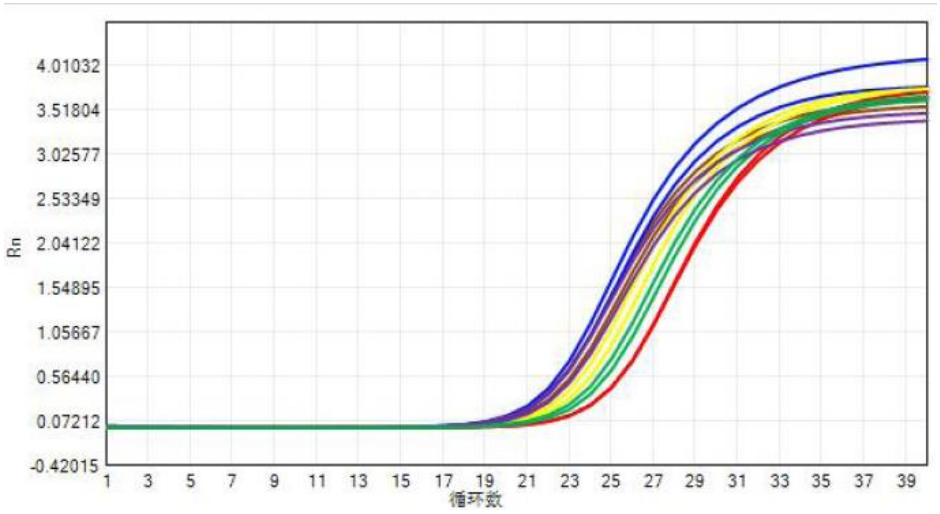


Figure 5. Fluorescent RT-PCR amplification curves for R4143 workflow compatibility testing.

6. Magnetic Bead-Based FFPE RNA Extraction
- IVD3022 MagPure FFPE RNA Kit

The MagPure FFPE RNA Kit (IVD3022) was evaluated as a magnetic particle-based FFPE RNA workflow. The kit uses high-binding magnetic particles, proteinase K digestion, DNase I treatment, magnetic washing, and elution. The workflow supports manual or liquid-station processing and can also be adapted to KingFisher Flex or similar magnetic extraction platforms.

5 mg one-year paraffin-embedded chicken liver and fish liver samples were processed using the MagPure FFPE RNA Kit. During extraction, different isopropanol volumes were used to support total RNA and small RNA recovery behavior. The data below are retained as magnetic bead-based FFPE RNA performance evidence for IVD3022.

Sample	Isoprop.	Conc. (ng/μL)	A260/280	A260/230	Yield (μg)
Chicken liver	0.7V	318.1	2.02	1.29	9.5
	0.7V	409.7	2.07	1.79	12.2
	1.5V	654.5	1.96	1.21	19.6
	1.5V	691.4	2.06	1.62	20.7
Fish liver	0.7V	277.4	1.86	0.95	8.3
	0.7V	195.2	2.06	1.43	5.8
	1.5V	371.1	1.83	0.87	11.1
	1.5V	255.7	2.07	1.08	7.6

From 5 mg FFPE chicken liver input, IVD3022 produced RNA yields of 9.5-12.2 μg under the 0.7V condition and 19.6-20.7 μg under the 1.5V condition. From 5 mg FFPE fish liver input, yields were 5.8-8.3 μg under the 0.7V condition and 7.6-11.1 μg under the 1.5V condition. Electrophoresis analysis supported recovery of FFPE RNA and showed that alcohol concentration can influence the relative recovery of larger RNA and small RNA fragments.