Performance Validation Report

HiPure Circulating DNA/RNA Kit (R4316)

1. Report Scope

This report summarizes internal validation data for HiPure Circulating DNA/RNA Kit (R4316). The kit is intended for recovery of circulating DNA, RNA and miRNA from serum, plasma and other cell-free liquid samples. The validation experiments used simulated plasma or water samples spiked with DNA marker, short DNA marker or viral RNA to evaluate recovery performance and RT-qPCR compatibility.

The workflow uses a two-stage column strategy for large-volume circulating nucleic acid processing: initial large-volume capture using a midi column, followed by micro-column concentration and elution. This report focuses on manual extraction results under the tested conditions.

2. Experiment 1: Low-Input DNA Recovery

Objective: To evaluate the recovery of low-input DNA using R4316 from a protein-containing plasma matrix and a low-background water matrix.

Matrix / Spike-In	Input	Recovered	Recovery	Method
Porcine Plasma + 500 ng DNA	1 mL	333.5 ng	86%	Qubit
Porcine Plasma + 500 ng DNA	1 mL	337.5 ng	87%	Qubit
Water + 20 ng DNA	1 mL	19.0 ng	94%	Qubit
Water + 20 ng DNA	1 mL	17.5 ng	86%	Qubit

In the plasma matrix, a relatively high amount of DNA marker was spiked to reduce the influence of endogenous background nucleic acids. In the water matrix, a lower DNA spike-in amount was used because background nucleic acid was absent. Under these conditions, the kit recovered 86-87% of DNA from plasma and 86-94% of DNA from RNase-free water. The results support efficient recovery of low-abundance DNA from both plasma-like and low-background sample matrices.

3. Experiment 2: Short-Fragment DNA Recovery

Objective: To evaluate recovery of short DNA fragments using 50 bp DNA marker (~3μg) spiked into porcine plasma. 1 ml of porcine plasma was mixed with Buffer CFL, incubated for nuclease inactivation, spiked with 50 bp DNA marker, and processed manually with R4316. The elution volume was 50 μL, and the purified DNA was analyzed by Nanodrop and agarose gel electrophoresis.

Recovered	Recovery	A260/A280	A260/A230
2.43 μg	81%	1.81	0.67
2.90 μg	97%	1.80	0.65
2.72 μg	91%	1.73	0.69
2.61 μg	87%	1.70	0.66

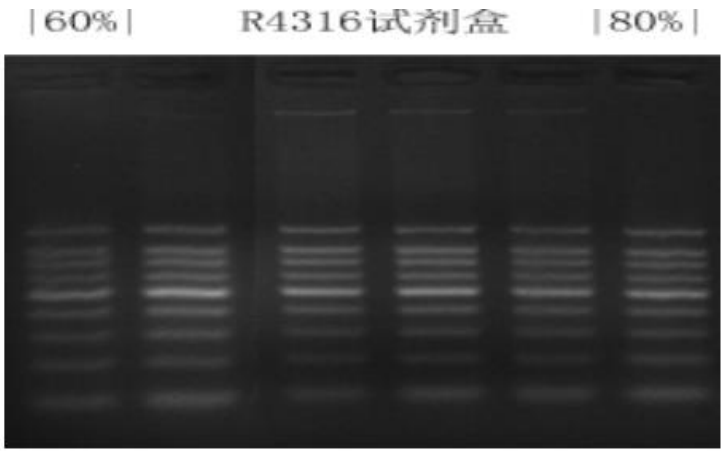

Figure 1. Agarose gel analysis of 50 bp DNA marker recovery from porcine plasma using R4316.

A260/A280 values were 1.70-1.81, indicating acceptable protein removal under the tested conditions. A260/A230 values were low, which was expected for this low-input spike-in test using guanidine-containing chemistry and was not used as the strict acceptance criterion for this experiment. Gel analysis showed visible recovery of the 50 bp DNA marker, with estimated short-fragment recovery above 80%. Calculated recovery values were 81–97% under the tested conditions.

4. Experiment 3: RT-qPCR Compatibility of Recovered Viral RNA

Objective: To evaluate whether R4316 recovery products are compatible with fluorescent RT-PCR. 1 µl of Newcastle disease virus RNA was spiked into porcine plasma or water samples and extracted using R4316. A reference viral nucleic acid workflow was tested in parallel. The R4316 workflow used 1 mL plasma or water input, while the reference viral workflow used 0.2 mL plasma or water input. The elution volume was 50 µL.

Workflow	Sample Matrix	Ct
R4316	Porcine plasma	19.52
R4316	Porcine plasma	19.60
R4316	Water	19.33
R4316	Water	19.37
Reference viral workflow	Porcine plasma	19.34
Reference viral workflow	Water	19.03

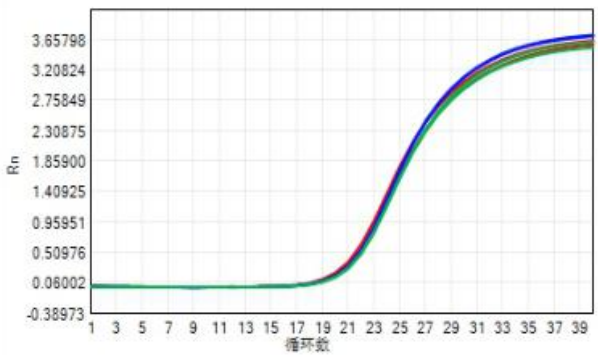

Figure 3. Fluorescent RT-PCR amplification curves of viral RNA recovered using R4316 and reference workflow conditions.

R4316 extracts from plasma produced Ct values of 19.52-19.60, while R4316 extracts from water produced Ct values of 19.33-19.37. The reference viral workflow produced Ct values of 19.34 for plasma and 19.03 for water. The close Ct values indicate that R4316 recovery products were compatible with RT-qPCR and did not show obvious plasma-derived inhibition in this spike-in test.

6. Summary

Across the tested simulated plasma and water sample systems, R4316 showed recovery rates above 80% for low-input DNA and short DNA fragments under the tested conditions. The workflow also produced RT-qPCR-compatible viral RNA recovery products, with Ct values close to those obtained using a reference viral nucleic acid workflow.

These data support R4316 as a circulating nucleic acid extraction workflow for plasma, serum and other cell-free liquid samples where DNA, RNA and miRNA recovery are required. Because the experiments used spike-in model systems, the results should be interpreted as recovery-supporting evidence under controlled test conditions rather than as a direct quantitative claim for every clinical sample type.