# Performance Characteristics

RNeasy® DSP FFPE Kit, Version 1 REF 73604

Version management

This document is the RNeasy DSP FFPE Kit Performance Characteristics, Version 1, R1.

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| CE IVD  | Check availability of new electronic labeling revisions at **www.qiagen.com/HB-2416** before test execution. The current revision status is indicated by the issue date (format: month/year). |

## General introduction

The RNeasy DSP FFPE Kit is intended for the purification of total RNA from formalin-fixed, paraffin embedded (FFPE) tissues.

The product is intended to be used by professional users, such as technicians and physicians that are trained in molecular biological techniques. It uses an optimized silica spin-column-based protocol, and includes enzymatic removal of residual DNA.

The RNeasy DSP FFPE Kit isolates RNA molecules longer than 70 nucleotides, and provides recovery of usable RNA fragments for downstream applications such as RT-PCR.

## Yield of purified RNA

The basic performance of the RNeasy DSP FFPE Kit was evaluated using FFPE samples from 5 different human tissues (breast, colon, lung, melanoma cancer and normal skin; 20 samples each).

FFPE samples may exhibit a high degree of tissue heterogeneity. In addition, tissue surface area is highly variable in FFPE samples, leading to variable quantity of extracted RNA. Therefore, the user should optimize the number of sections, section thickness and section surface area for their sample of interest and any procedures used in their laboratory.

If the kit is being used in conjunction with a QIAGEN® downstream application, refer to the relevant handbook for instructions.

Insufficient tissue dehydration during FFPE tissue preparation, adding too much paraffin to the sample in the extraction tube, using lower-purity ethanol (not molecular-biology-grade) than recommended or retaining ethanol in the sample may lead to suboptimal extraction and low RNA quantity or reduced downstream performance.

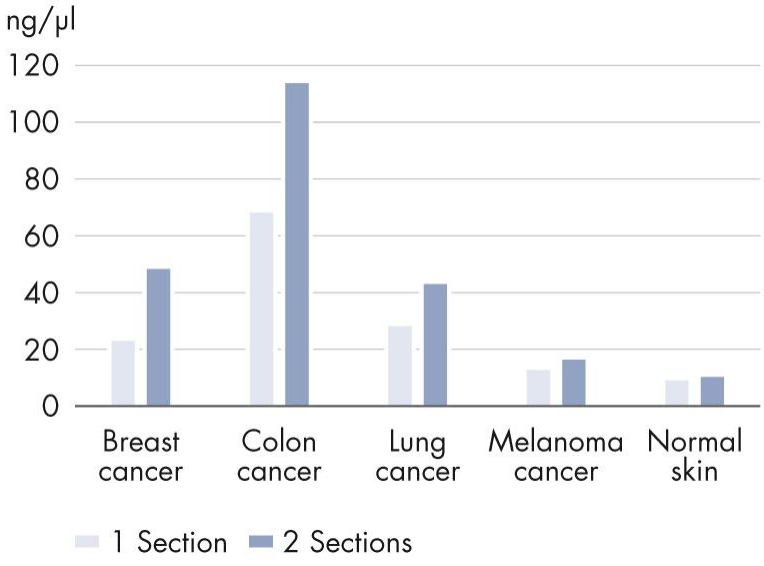


**Figure 1. RNA yields from different human tissues (32 µl elution volume).**

## Downstream analysis

Eluted RNA is ready for use in downstream assays. To evaluate performance, 10 ng of RNA was isolated with the RNeasy DSP FFPE Kit from 5 different human tissues (breast, colon, lung, melanoma cancer and normal skin; 20 samples comprising of one or two sections) and validated using RT-PCR targeting the human β-actin gene. Amplification was successful, showing that RNA isolated with the RNeasy DSP FFPE Kit can be used for downstream analysis.

The user should optimize the number of sections, section thickness and section surface area for their sample of interest and any subsequent procedures used in their laboratory or refer to the specific performance of the relevant downstream assay.

| | Breast cancer | Colon cancer | Lung cancer | Melanoma cancer | Normal skin |
|---|---|---|---|---|---|
| RT-PCR 1 section | ✓ | ✓ | ✓ | ✓ | ✓ |
| RT-PCR 2 sections | ✓ | ✓ | ✓ | ✓ | ✓ |

**Figure 2. Successful RT-PCR amplification of 10 µm FFPE sections derived for five different human tissues tested.**

## Eluate stability

Eluate stability will depend on the content and type of co-purified impurities (related to tissue type), elution volume and storage conditions. We recommend that users establish the eluate stability as needed for their particular requirements.

Eluate stability was tested for FFPE-derived human RNA samples stored at –15 to –30°C and –60 to –90°C. No deterioration was observed for up to 12 weeks and eluates stored at room-temperature (18–25°C) were stable for up to 12 hours. All conditions were assessed using RT-PCR targeting the human β-actin gene.

If the kit is being used in conjunction with QIAGEN downstream applications, refer to the relevant kit handbook for instructions.

## Repeatability

Repeatability was evaluated using FFPE sample of nucleated human blood cells. The samples were tested with an internally validated assay for a 295 bp fragment of the human β-actin gene on an ABI® 7900 real-time PCR cycler.

For statistical analysis, 108 data points from three extraction batches (same kit lot, operator, day) were used. Statistical analysis included the calculation of the standard deviation (SD) and coefficient of variation (CV) of the $C_T$ values derived from the β-actin RT-PCR. The SD was 1.1 $C_T$ and the coefficient of variation was 4.1% (Table 1).

**Table 1. Repeatability results**

| Repeatability | | | |
|---|---|---|---|
| | Mean $C_T$ | SD | CV (%) |
| Batch 1 | 26.64 | 1.01 | 3.81 |
| Batch 2 | 27.51 | 1.16 | 4.2 |
| Batch 3 | 27.23 | 0.95 | 3.5 |
| Batch 1 + 2 + 3 | 27.13 | 1.11 | 4.07 |

## Reproducibility

Reproducibility was performed by assessing RNA extractions from FFPE samples of nucleated human blood cells with different operators, on different days and different operators and days. The samples were tested with an internally validated assay for a 295 bp fragment of the human β-actin gene on an ABI 7900 real-time PCR cycler. For statistical analysis, 108 data points from three extraction batches were used for each test setting. Statistical analysis included the calculation of the standard deviation (SD) and coefficient of variation (CV) of the $C_T$ values derived from the β-actin RT-PCR (Table 2).

**Table 2. Reproducibility results**

| Reproducibility | | | |
|---|---|---|---|
| | Mean $C_T$ | SD | CV (%) |
| Different operators | 26.92 | 1.06 | 3.95 |
| Different days | 26.56 | 1.20 | 4.53 |
| Different operators and days | 26.63 | 1.01 | 3.78 |

## Linearity

The RNeasy DSP FFPE Kit can be used for isolation of RNA from different FFPE tissue types. The system was validated for the use of 1–4 sections from FFPE nucleated human blood cells and showed a linear increase in RNA yield. A linear range should be established as per customer requirements and validated for the particular use. Different linear ranges are expected for different tissue types, depending on the tissue load into the system, as well as tissue characteristics and downstream assays.

## Interfering substances

Potentially interfering substances can originate from different sources, e.g., natural metabolites specific for the tissue type and organ, metabolites produced during pathological conditions, substances introduced during patient treatment or substances ingested by the patient. Due to the complexity of potential interfering substances and different sensitivity of specific downstream applications, we recommend that users assess the effect of the interfering substances for their own systems and validate a method for controlling interference in their specific diagnostic downstream application.

Interfering substances derived from components of the RNeasy DSP FFPE Kit during sample processing and RNA extraction were not observed.

For more information on interfering substances in specific QIAGEN downstream applications, refer to kit handbooks.

## Cross-contamination

To assess the level of cross-contamination, 500 ng total RNA from blood were spiked into the deparaffinization solution and isolated adjacent to tubes not containing RNA (extraction negative tubes). The study aimed to mimic the situation whereby samples containing a high level of RNA target molecules can cross-contaminate other samples during the extraction procedure. RNA purification was conducted using one lot of reagents. Cross-contamination was assessed using RT-PCR targeting the human β-actin gene. The results showed no cross-contamination within the entire system.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.