

DropSense16 App – RNA FFPE

Quantification of RNA from FFPE samples using cDrop



Fig 1. App button on the DropSense16 app selection screen

RNA Extraction kit	Brand	Type
PureLink FFPE RNA	Life Technologies	Column
Nugen Prelude FFPE RNA	NuGEN	Column
NucleoSpin total RNA FFPE	Macherey - Nagel	Column
QIAasympohy RNA kit	Qiagen	Beads

Table 1. Overview of used extraction kits



Fig 2. Illustrations of the results screen on DropSense16.

Introduction

An app for specific quantification of total RNA extracted from FFPE tissue sections is available on DropSense16. Trinean's proprietary cDrop software is used to isolate the profile of the desired molecule (here RNA) from the measured UV/VIS spectrum, thereby distinguishing it from absorbing sample contaminants. The specific RNA profile is then used to determine the RNA concentration. In this note we describe how to use this application and how to interpret its results.

App description

This cDrop app is specifically designed to quantify RNA extracted from formalin-fixed paraffin-embedded tissue sections. This app is developed using RNA extracted from liver, lung, lymph, spleen and uterus tissue blocks. Three column-based extraction methods and one bead-based method (see table 1) were used for RNA isolation. For proper use of this application, always use pure water as blank(s) on the DropSense16 slides.

App profiles

The cDrop algorithm in this app will analyse the measured UV/VIS spectrum (white curve) to extract specific profiles (see figure 2):

- **RNA (blue)** = molecule of interest
- **Impurities (orange)** = molecules except RNA with absorbance in UV/VIS. These are: degraded RNA (nucleotides), dsDNA, degraded DNA (dNTP's), proteins, guanidine-thiocyanate, azide, phenol, xylene, NP-40, Triton X-100 and citrate
- **Background (grey)** = profile combining intrinsic sample turbidity, scattering from bead contamination and haemoglobin/haeme residue (if present, absorbance max visible at 405 nm). Background is subtracted from the measured spectrum prior to cDrop analysis.
- **Residue (Yellow)** = unidentified part of the measured spectrum, also shown as % of the measured spectrum. This is a cDrop quality control parameter.

In certain cases, a measurement doesn't allow accurate discrimination of RNA: **(1)** samples with nucleic acid content below 0.5 OD (A260, 10mm) after background correction or **(2)** samples with residue above 2,5% due to an unknown component. In these circumstances, a total nucleic acid spectrum (pink spectrum, figure 2) will be displayed instead of the RNA spectrum. Concentration is calculated using the A260 value of this profile multiplied by the concentration factor of RNA (=40).

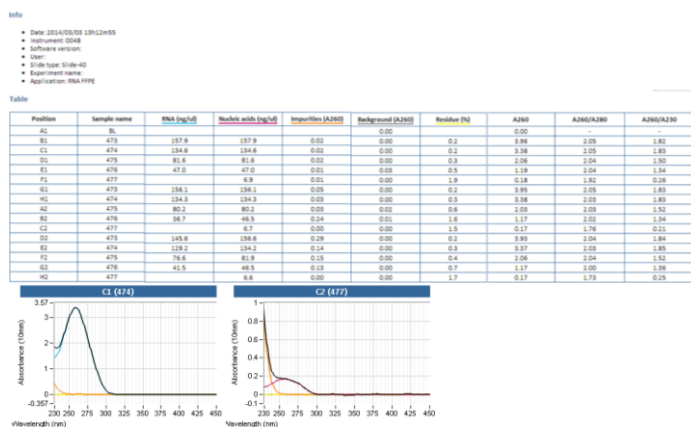


Fig 3. Example of HTML report

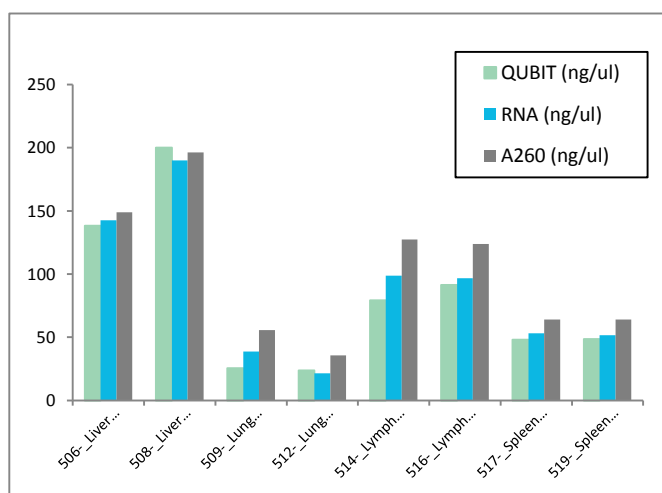


Fig 4. DropSense16 data compared to Qubit RNA assay and A260

App Report

Four report types are generated: an HTML file, XML, txt and a CSV file (for import in MS Excel). All reports include a table containing all cDrop profiling results as well as classic spectrometry data like A260, 260/230 and 260/280 (It should be noted that these values are calculated after subtraction of the background profile). In addition, for each separate measurement, the HTML report contains graphs with the profiling curves. Headings of the table columns are underlined with the colours matching the profile in the graphs (see figure 3).

These reports are easily exported from DropSense16 to a USB key, network folder or to a smart device (using the QR code). An additional DropSense16 specific measurement file (.bin) is also exported.

Results

Figure 4 shows comparative data for a subset of samples used to develop the RNA FFPE application. Total RNA was extracted from 4 different tissues, using the QIA Symphony RNA kit. DropSense16 data was compared to Qubit (Qubit™ RNA HS Assay) and A260. DropSense16 data (blue bars) show close resemblance to the RNA specific Qubit method (green bars), while A260 has the tendency to overestimate RNA concentration (grey bars).

Conclusion

The DropSense16 RNA FFPE app can specifically quantify the RNA content of FFPE RNA extractions, by analysing the UV/VIS spectrum directly from the sample without the need for an assay or dilution steps.



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