Quantification of total nucleic acids from unknown sources (RNA eq)

Introduction

In this note, we describe how to use the Nucleic Acids (RNA equiv.) application on the Lunatic systems. This Unmix application is used to analyze the UV/Vis spectral shape of the sample to isolate the fraction of the molecule of interest from co-absorbing entities contributing to the total UV/Vis absorption spectrum. Accurate quantification of the molecule of interest is established using its isolated spectrum fraction. This application does not distinguish RNA from DNA, but rather isolates the total nucleic acid concentration in RNA extractions from unknown sources (bacterial, yeast, plant, plasmid, etc.).

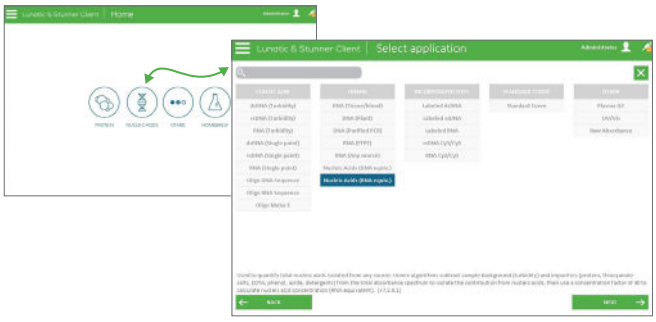

Figure 1: Illustration of the Lunatic "Select application" interface. The image in the back shows the Sample Type screen, whereas the image in the front displays the applications available for the selected Sample Type.

App selection

On the Lunatic, the Nucleic Acids (RNA equiv.) application can be found in the "Unmix" column upon selection of "Nucleic Acids" in the Sample Type screen (Figure 1). On the Little Lunatic, this application can be found on the applications screen selecting RNA as sample type (Figure 2). In case no sample type on the Little Lunatic is defined, check the description to assure the app calculates concentrations in RNA equivalents. Aside from sample names, no additional user input is required. For proper use of Unmix applications, always use pure water as blank(s).

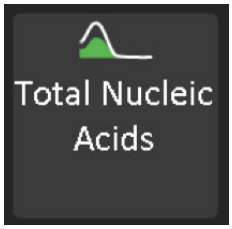

Figure 2: App button on the Little Lunatic app selection screen.

Results on screen

The Unmix app will analyze the measured UV/Vis spectrum to detect the presence of specific component groups (Figures 3 and 4):

- **Nucleic acids (purple)**: molecule of interest in ng/µL RNA equivalent. This profile is specific for a wide variety of nucleic acids (total RNA, degraded RNA, DNA with 25%-75% GC, etc.). The concentration is calculated using the A260 peak value of this profile multiplied by the concentration factor of RNA (= 40).

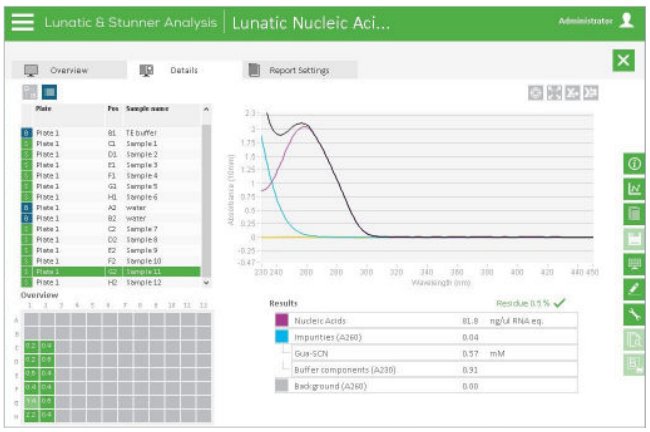

Figure 3: Illustration of the Results screen on the Lunatic. Unmix app results of the selected sample are shown as spectral shape as well as in calculated values. Additional impurity and/or background detailing will be reported if possible.

- **Impurities (blue)**: non-nucleic acid molecules that also absorb in the UV/Vis-region, reported in OD260 values. Additional impurity detailing is reported if thresholds are exceeded (Table 1).
- **Background (gray)**: sample turbidity profile, reported in OD260 values. The background spectrum is subtracted from the measured spectrum, resulting in the content spectrum (black curve on the Lunatic, white curve on the Little Lunatic). Additional background detailing is reported if thresholds are exceeded (Table 2).

The residue or 'Quality of fit' value (RRSE) is the % of the measured spectrum which could not be annotated, representing the quality of fitting. This parameter is displayed as a yellow curve as well as a percentage value below the graph. A warning sign (red cross) will appear for samples with a residue value above 2.5% due to (1) too high turbidity of the sample, (2) presence of an unknown chemical, (3) low-concentrated samples. When this warning sign appears or when samples have an A260 below 0.5 OD, the additional impurity/background detailing will not be shown.

Report

A variety of report types are generated: an HTML, XML, TXT and a CSV file are created on both systems. In addition, the Lunatic also creates XLSX and PDF report files. On the Little Lunatic fixed report templates are used while the larger Lunatic system allows full flexible selection of the content to be reported.

Figure 4: Illustration of the Results screen on the Little Lunatic. Unmix app results of the selected sample are shown as spectral shape as well as in calculated values. Additional impurity and/or background detailing will be reported if possible.

Impurity	Threshold	Reported in
Phenol	0.086 mM	mM
Thiocyanate salts	0.1525 mM	mM
Buffer components	0.5 OD230	OD230
Detergents	0.1 OD260	OD260
Protein	0.1 OD280	OD280

Table 1: Additional impurity detailing reported if thresholds are exceeded.

Background	Threshold	Reported in
Beads	0.5 OD260	OD260
Heme	0.05 OD260	OD260
Chlorophyll	0.1 OD260	OD260

Table 2: Additional background detailing reported if thresholds are exceeded.

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