

DropSense16 Apps - RNA Cy3/Cy5 and ssDNA Cy3/Cy5

Quantification of Cy3/Cy5 labeled RNA and ssDNA using cDrop



Fig 1. App buttons on the DropSense16 app selection screen

Introduction

An app for specific quantification of Cy3/Cy5 labeled RNA or ssDNA is available on DropSense16. Trinean's proprietary cDrop software is used to isolate the RNA or ssDNA profile as well as specific Cy3 and Cy5 profiles from the measured UV/VIS spectrum, thereby distinguishing them from other absorbing sample contaminants. These profiles (RNA or ssDNA + Cy3 and Cy5) are then used to determine the total RNA or ssDNA concentration plus amount of Cy3 and Cy5 label. In this note we describe how to use this application and how to interpret its results.

App description

These cDrop apps are specifically designed to determine the degree of labelling (DoL) of Cy3/Cy5 labeled RNA or ssDNA samples. For proper use of this application, always use pure water as blank(s) on the DropSense16 slides.

App profiles

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

- **ssDNA or RNA** (blue) = molecule of interest (depending on app)
- **Cy3** (green) = Cy3 label present in sample
- **Cy5** (red) = Cy5 label present in sample
- **Impurities** (orange) = molecules except RNA or ssDNA with absorbance in UV/VIS. These are: Proteins, guanidine-thiocyanate, azide for both applications, for the RNA application phenol is included as are EDTA and citrate for the ssDNA application.
- **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. For samples having a residue above 2.5%, a red cross will be displayed on screen.

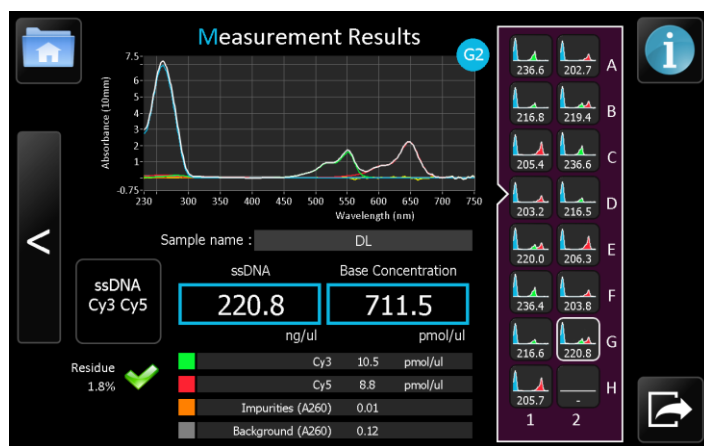


Fig 2. Illustrations of the results screen on DropSense16.

Position	Sample name	ssDNA (ng/ul)	Base Concentration (pmol/ul)	Cy3 (pmol/ul)	DoL_Cy3 (%)	Cy5 (pmol/ul)	DoL_Cy5 (%)	Impurities (A260)
A1	Cy3	236.6	762.5	20.7	2.72	0.1	0.01	0.01
B1	Cy3 1/2	216.8	699.1	10.0	1.43	0.0	0.00	0.01
C1	Cy5	205.4	660.2	0.2	0.02	17.3	2.63	0.01
D1	Cy5 1/2	203.2	654.0	0.1	0.01	8.6	1.31	0.01
E1	DL	220.0	708.5	10.4	1.47	8.7	1.23	0.01
F1	Cy3	236.4	761.3	20.7	2.72	0.0	0.00	0.01
G1	Cy3 1/2	216.6	698.6	10.0	1.44	0.0	0.00	0.01
H1	Cy5	205.7	660.0	0.2	0.03	17.3	2.62	0.02
A2	Cy5 1/2	202.7	651.8	0.2	0.03	8.7	1.34	0.01
B2	DL	219.4	706.5	10.4	1.47	8.7	1.23	0.01
C2	Cy3	236.6	762.9	20.7	2.71	0.0	0.00	0.01
D2	Cy3 1/2	216.5	698.9	10.0	1.43	0.0	0.01	0.00
E2	Cy5	206.3	662.4	0.2	0.03	17.4	2.62	0.02
F2	Cy5 1/2	203.8	655.0	0.1	0.02	8.5	1.30	0.01
G2	DL	220.8	711.5	10.5	1.47	8.8	1.23	0.01
H2	BL	-	-	-	-	-	-	-

Background (A260)	Residue (%)	A260	A550	A650
0.00	1.4	7.49	3.11	-0.00
0.00	1.6	6.79	1.52	0.01
0.35	2.0	6.81	0.25	4.34
0.27	1.9	6.54	0.13	2.18
0.17	1.5	7.15	1.69	2.18
0.04	1.4	7.52	3.11	-0.01
0.00	1.7	6.82	1.51	0.00
0.40	2.4	6.85	0.25	4.31
0.34	2.0	6.54	0.14	2.17
0.04	1.6	7.14	1.69	2.19
0.00	1.4	7.51	3.12	-0.01
0.00	1.9	6.84	1.51	0.06
0.20	2.4	6.87	0.25	4.29
0.29	1.8	6.55	0.13	2.14
0.12	1.8	7.19	1.70	2.20
0.04	-	0.00	0.00	0.00

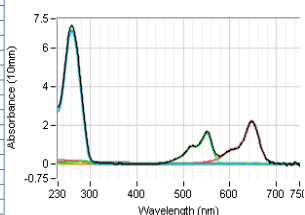


Fig 3. Example of HTML report

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 results as well as classic spectrometry data like A260 (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 cDrop curves. Headings of the table columns are underlined with the colours matching the profile in the graphs (see figure 3). These reports can be 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

Information which can be retrieved from any of the above described reports:

- RNA or ssDNA concentration (ng/μl)
- total base concentration (pmol/μl)
- Cy3 (pmol/μl) and Cy5 (pmol/μl)
- Degree of labelling (DoL) in %, Cy3/Cy5 (pmol/μl) divided by total base concentration, multiplied by 100
- impurities, Background and Residue
- A260, A550 and A650

Discussion

The DropSense16 Cy3/Cy5 RNA or Cy3/Cy5 ssDNA app can specifically quantify the RNA or ssDNA content and Degree of labeling, by analysing the UV/VIS spectrum directly from the sample without the need to run an assay or to dilute the samples. In addition, the export tables provide traditional A260 results plus impurity information.



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