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1.0 Purpose

This procedure describes the materials and methods required for operation of the Qubit™ 4 Fluorometer from Invitrogen.

2.0 Scope

This SOP applies to BDP personnel operating the Qubit™ 4 Fluorometer from Invitrogen. This SOP describes the procedures used for the basic operation and maintenance of the Qubit™ 4 Fluorometer. Assay-specific protocols will be included as attachments for the use of this system in detecting and quantifying nucleic acids and proteins present in test samples.

3.0 Authority and Responsibility

3.1 The Director, Process Analytics/Quality Control (PA/QC) has the authority to define this procedure.

3.2 PA/QC is responsible for training laboratory personnel and documenting this training to Biopharmaceutical Quality Assurance (BQA).

3.3 PA/QC personnel are responsible for the performance of this procedure.

3.4 PA/QC is responsible for reviewing the data and documentation of the results of this procedure.

3.5 BQA is responsible for quality oversight of this operation.

4.0 Equipment, Materials, and Reagents

4.1 Qubit™ 4 Fluorometer Instrument (Invitrogen)

4.2 Qubit™ 4 System Verification Assay Kit (BDP PN 31309)

4.3 Qubit™ Assay Tubes (BDP PN 31306)

4.4 Qubit™ Assay Kit – see assay specific SOP attachment(s) for details

4.5 1X TE (BDP PN 30267 or BDP-approved equivalent), Nuclease Free water (BDP PN 10189 or BDP-approved equivalent), or PBS Dilution Buffer (BDP PN 30007 or BDP-approved equivalent), as needed, for making sample dilutions – see assay specific SOP attachment(s)

4.6 BDP-approved Aerosol Barrier Tips as Needed (1000uL BDP PN 21471, 200uL BDP PN 21470, or 20uL BDP PN 21472 or BDP-approved equivalent)

4.7 BDP-approved Powder-free Gloves as Needed (Large BDP PN 20764, Medium BDP PN 20765, or Small BDP PN 20766 or BDP-approved equivalent)

4.8 Microcentrifuge Tube, Low Binding, 1.7 mL (BDP PN 31129 or BDP-approved equivalent) or 15 mL Centrifuge Tube (BDP PN 20006 or BDP-approved equivalent) for making Qubit™ Working Solution

4.9 Low-lint polyester wipes (clean-room grade)

5.0 Procedure

5.1 Instrument Overview

5.1.1 External Components (Please see Attachment 1)

5.2 Device Verification/Calibration Test

NOTE: This should be performed yearly and documented in the instrument logbook.

5.2.1 On the Home screen, press Settings. 

5.2.2 On the Settings screen, press Device verification test.

5.2.3 Assay Setup

5.2.3.1 Include all reagents on Form 22975-01.

5.2.3.2 Label three Qubit™ Assay Tube lids: 1-3.

5.2.3.3 Add 200 ul of Blank Reagent to Tube 1.

5.2.3.4 Add 200 ul of Green Fluorescence Reagent to Tube 2.

5.2.3.5 Add 200 ul of Far Red Fluorescence Regent to Tube 3.

5.2.4 Press the Start Test button and follow instructions.

5.2.5 Record the results on Form 22975-01.

5.2.5.1 If the verification failed, it may be repeated. If the verification fails again place the fluorometer out of service and contact ThermoFisher.

5.3 Perform Assay

NOTE: See assay specific attachments for appropriate method to quantify samples (DNA, RNA, Protein)

5.3.1 Assay Standardization for valid assay

5.3.1.1 On the Home screen, select the assay type for which you wish to read new standards.

5.3.1.2 Add reagents information to Form 22975-02

5.3.1.3 Select the desired assay.

5.3.1.4 Press the read standards button

5.3.1.5 At the prompt, insert the proper Standard into the sample chamber and press Read standard.

NOTE: Be sure to use the Standard that is appropriate for the assay (see assay specific attachment) you are performing.

5.3.1.6 If the standardization is successful, the software displays the *Read standard* screen with the *Fluorescence vs. Concentration* graph.

5.3.1.6.1 Note on form 22975-03 that the standard passed

NOTE: In the Fluorescence vs. Concentration graph, the standard data points are connected by a line and open circles represent correct standards.

5.3.1.7 If the standardization is not successful, the software displays the *error* message

5.3.1.7.1 In the Error screen, press OK.

5.3.1.7.2 Rerun the samples

5.3.1.7.3 If error persists then remake the standard as described on the assay specific protocols.

NOTE: For the Qubit™ dsDNA BR, Qubit™ dsDNA HS, Qubit™ ssDNA, Qubit™ microRNA, Qubit™ RNA HS, Qubit™ RNA BR, Qubit™ RNA XR, and Qubit™ protein assays, the reading given by Standard #2 should be at least ten times higher than that of Standard #1.

NOTE: For the Qubit™ protein assay, the reading given by Standard #3 should be at least 40% higher than that of Standard #2.

5.3.2 Read Samples

5.3.2.1 Prepare samples according to assay-specific instructions (see Attachment).

NOTE: Incubate the samples for the appropriate amount of time after mixing them with the working solution (2 minutes for the Qubit™ DNA and RNA assays, 15 minutes for the Qubit™ protein assay).

5.3.2.2 On the Home screen, select the assay type for which you wish to read new standards.

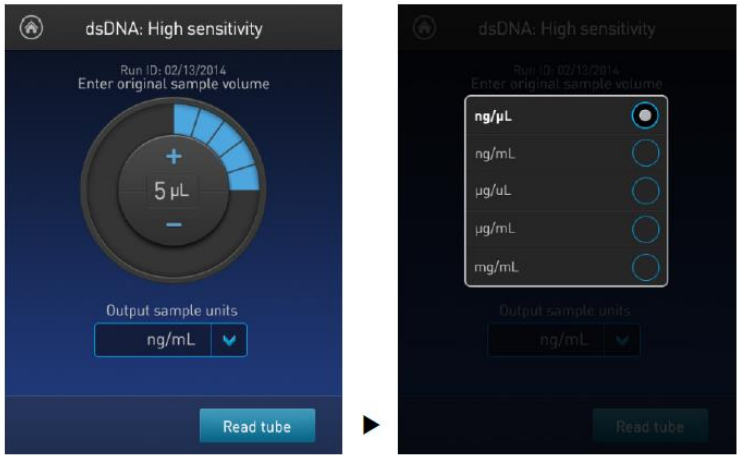
5.3.2.3 Select the desired assay.

5.3.2.4 Press the run sample button

5.3.2.5 In the *Sample volume* screen, select the sample volume and units for the Qubit™ quantitation assays or the Qubit™ RNA IQ assay:

5.3.2.5.1 Press the + or – buttons on the wheel to select the sample volume added to the assay tube (between 1 and 20 µL).

5.3.2.5.2 For the Qubit™ quantitation assays, select the units for the output sample concentration from the drop-down menu.



5.3.2.6 Insert a sample tube into the sample chamber, close the lid, and then press Read tube

5.3.2.7 Remove the current sample and insert a new sample.

5.3.2.7.1 To change the sample volume, swipe right or press the left arrow.

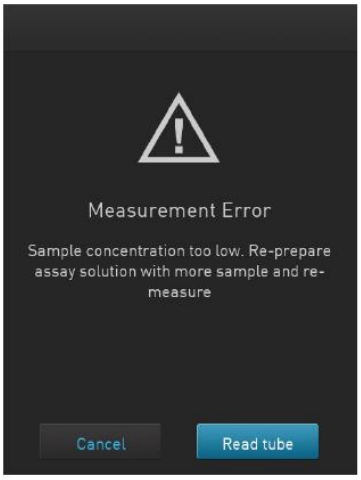
5.3.2.8 Press Read tube.

5.4 Results

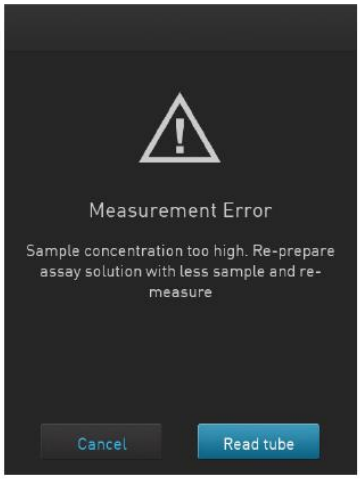
5.4.1 The Results will display after each sample is read.

5.4.1.1 If the results are within the assay's range, the concentration values are displayed. The top value (in large font) is the concentration of the original sample and the bottom value is the dilution concentration.

5.4.1.2 If the results are outside of the assay's range, an "Out of Range" message is displayed.



Measurement error – Sample concentration too low



Measurement error – Sample concentration too high

5.4.1.2.1 If an error message appears, adjust the sample dilutions accordingly and re-analyze.

5.5 Manage Data

5.5.1 View Sample data

5.5.1.1 From the Concentration, Graph, RNA IQ results, or Home screen, press Data.

5.5.1.2 On the Export data screen, press the data set of interest

5.5.1.3 To view the sample details, press the sample of interest. (data details screen)

5.5.2 Rename Data Files

5.5.2.1 From the Concentration, Graph, RNA IQ results, or Home screen, press Data.

5.5.2.2 On the Export data screen, press the data set of interest

5.5.2.3 To view the sample details, press the sample of interest. (data details screen)

5.5.2.4 On the Data details screen, press the Sample # field (indicated by the red arrow), enter the new name using the keyboard that appears, then press Enter.

5.5.3 Export Data

5.5.3.1 Insert the USB drive into the Qubit™ 4 Fluorometer.

5.5.3.2 On the Concentration, Graph, RNA IQ results, or Home screen, press Data.

5.5.3.3 On the *Export data* screen, check the selection box to the left of each data set you wish to export.

5.5.3.4 To save only individual data entries from a data set, press the data set of interest, and then check the selection box to the left of the samples you wish to export.

5.5.3.5 Press Export to export the data. The numeric data is automatically saved as a CSV file.

5.5.3.6 Transfer the USB drive to the USB drive port on your computer. Open the CSV file using any spreadsheet program.

5.5.3.7 Save CSV file to S Drive and add location and data to Form 22975-03.

5.5.4 Delete Data (Only delete data that has been saved on the S Drive)

5.5.4.1 On the Concentration, Graph, RNA IQ results, or Home screen, press Data.

5.5.4.2 On the *Export data* screen, check the selection box to the left of each data set you wish to delete.

5.5.4.3 To delete only individual data entries from a data set, press the data set of interest, and then check the selection box to the left of the samples you wish to delete.

5.5.4.4 Press Delete. A warning screen appears.

5.5.4.5 Press Delete to permanently delete the sample data or data set once saved on S Drive.

5.6 Instrument Settings/Configurations

5.6.1 On the Home screen, press Settings. 

5.6.2 On the Settings screen, press About Instrument to display the About Instrument screen.

5.6.3 Sleep mode

5.6.3.1 On the *Instrument settings screen* (5.6.2), press Sleep mode to display the *Sleep mode* screen.

5.6.3.2 Enter the time in minutes allowed before the instrument goes into sleep mode by pressing the minutes field, then using the number pad to select a value during initial setup.

NOTE: The software requires a minimum of 1 minute and a maximum of 60 minutes.

5.6.3.3 Press Done to save the changes and return to the Instrument settings screen.

5.6.4 Date/Time Adjustment

5.6.4.1 On the *Instrument settings screen* (5.6.2), press Date/Time.

5.6.4.2 Select a date format, select a time format, then press Next.

5.6.4.3 Enter or changing the date and time:

5.6.4.3.1 Press a date field (day, month, or year), then use the number pad to select a value.

5.6.4.3.2 Press a time field (hours or minutes), then use the number pad to select a value.

5.6.4.3.3 Select AM or PM.

5.6.4.4 Press Done to save the changes and return to the Instrument settings screen.

6.0 Documentation

Record all the reagent part numbers, lot numbers, expiration dates, and results for the yearly calibration on Form 22975-01 (attachment 2) and submit to BRAM. Record all reagent part numbers, lot numbers and expiration dates for the experiment on Form 22975-02 (Attachment 2). Record the results on Form 22975-03 (Attachment 3).

7.0 References and Related Documents

Form 22975-01 Qubit™ Validation/Calibration Form

Form 22975-02 Qubit™ Reagents and Sample Dilutions

Form 22975-03 Qubit™ Data Form

Qubit™ 4 Fluorometer User Guide, Revision C.0, Publication Number MAN0017209, Invitrogen, April 9, 2018.

8.0 Attachments

8.1 Attachment 1 External Components Figure

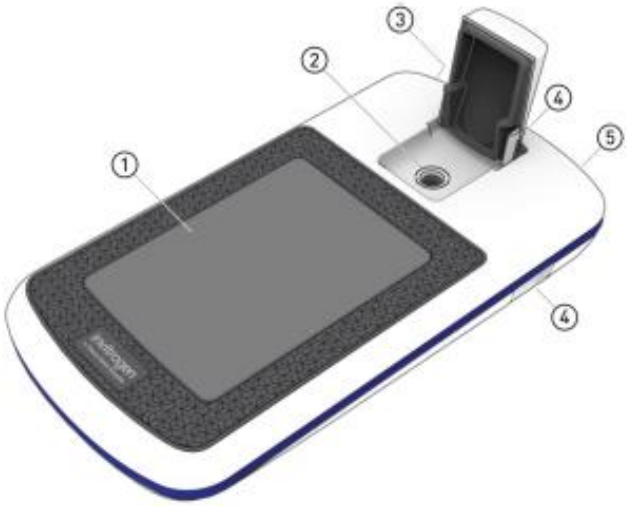
8.2 Attachment 5 Qubit® dsDNA HS Assay Kit Protocol

9.0 Change Summary

Attachment 1

External Components

Top view



Back view

1. **Touchscreen** is the user interface containing the controls for all the functions needed and displays data from the assays.
2. **Sample chamber** is used to load the assay tube containing the sample into the fluorometer for analysis.
3. **USB cable port (type mini-B)** allows you to transfer your data directly to your computer using the USB cable supplied with the instrument or any other similar USB cable.
4. **USB drive ports (Type A)** allow you to transfer and save data to your computer using the USB flash drive supplied with the instrument (or any other similar USB drive) for record keeping and printing purposes.
5. **Power inlet** connects the Qubit™ 4 Fluorometer to an electrical outlet using the supplied power cord and the appropriate plug, based on the electrical outlet configuration in your country.

Attachment 2
dsDNA HS (High Sensitivity) Assay Kit protocol

1.0 Equipment, Materials and Reagents

1.1 Qubit® dsDNA HS (High Sensitivity) Assay Kit (BDP PN 31307)

1.2 Qubit® Assay Tubes (BDP PN 31306)

1.3 Microcentrifuge Tube, Low Binding, 1.7 mL (BDP PN 31129 or BDP-approved equivalent) or 15 mL Centrifuge Tube (BDP PN 20006 or BDP-approved equivalent)

1.4 1X TE (BDP PN 30267 or BDP-approved equivalent)

2.0 Procedure

2.1 Store the Qubit® dsDNA HS Reagent and Buffer at room temperature and store the DNA standards at 2-8°C. For optimal performance, bring standards that are included in the kit to room temperature before use. Record the part number, lot number and expiry information for the Qubit dsDNA HS Assay kit on Form 22975-02.

2.2 Set up and label one Qubit® Assay tube for each sample to be measured as well as one Qubit® Assay tube for each of the two standards. Record the part number, lot number and expiry information for the Qubit Assay tubes on Form 22975-02.

NOTE: Do not label the sides of the Qubit® Assay tubes as this could interfere with the sample read.

2.3 Dilute the Qubit® dsDNA HS Reagent 1:200 in Qubit® dsDNA HS Buffer to prepare a Qubit® working solution. Record the volumes used to make the Qubit working solution on Form 22975-02.

2.3.1 The final volume in each tube (standard or sample) is 200 µL.

2.3.2 Each standard tube will require 190 µL of Qubit® working solution while each sample tube will require between 180 µL and 199 µL of Qubit® working solution.

2.3.3 Prepare a sufficient volume of Qubit® working solution for all samples and standards.

NOTE: Do not mix the working solution in a glass container.

2.4 Add 190 µL of Qubit® working solution to each of the standards tubes.

2.5 Add 10 µL of each standard to the appropriate tube containing Qubit® working solution and vortex for 2 to 3 seconds, being careful to avoid the introduction of bubbles.

2.6 Incubate the sample at room temperature for 2 minutes ± 10 seconds.

2.7 Proceed to the Assay Standard section 5.3.1 of SOP 22975 for calibration procedure.

2.8 For each sample to be tested (not standards), add a volume of Qubit® working solution so that the total volume of sample and working solution is 200 µL.

2.9 Add an appropriate volume of sample (between 1 µL and 20 µL) to the correct tube with Qubit® working solution.

2.10 Vortex each sample for 2 to 3 seconds and incubate at room temperature for 2 minutes ± 10 seconds. Record incubation start/stop times on Form 22975-02.

2.11 Proceed to the Read Samples section 5.3.2 of SOP 22975 for the steps needed to analyze the samples.