**Qubit® 3.0 Fluorometer Instrument Procedure**

1. **PRINCIPLE:**
   1. The determination of nucleic acid and protein concentrations can be achieved using the fluorometry method. Fluorometry, which is performed by a fluorometer, measures the parameters of fluorescence: its intensity and wavelength distribution. The parameters are used to identify and characterize the presence and the amount of specific nucleic acid or protein in a sample. The target molecule is identified and characterized by the wavelength of the fluorescent light it emits when excitation occurs.
   2. The Qubit® 3.0 Fluorometer (Qubit®) is a benchtop instrument used for the quantitation of DNA, RNA, microRNA, and protein using the highly sensitive and accurate fluorescence-based Qubit® quantitation assays. Nucleic acid is quantified in less than 5 seconds per sample.
   3. The fluorometer has a large color touch screen, which displays the user interface for easy workflow navigation.
   4. See Table 1 for the Qubit® 3.0 Fluorometer specifications for fluorescent detection:

**Table 1: The Qubit® 3.0 Detection Specifications**

|  |  |  |  |
| --- | --- | --- | --- |
| **Light Source Color** | **Excitation filters** | **Emission filters** | **Quantitation Assays Supported** |
| Red LED  (max ~635nm) | Red  600-645 nm | Red  665-720 nm | dsDNA, RNA, oligo (ssDNA), protein, and Ion Sphere |
| Blue LED  (max ~470nm) | Blue  430-495 nm | Green  510-580 nm | dsDNA, RNA, oligo (ssDNA), protein, and Ion Sphere |

* 1. The components of the Qubit® are as follow:
     1. **Power inlet** – connects the Qubit® 3.0 Fluorometer to an electrical outlet.
     2. **Touch screen** – the user interface to control all functions and display data.
     3. **Sample chamber** – used to insert the sample tube for quantitation.
     4. **USB drive port** – allows transfer of data for record keeping.
     5. **USB cable port** – allows transfer of data directly to a computer using the USB cable.
  2. Figure 1 displays the Qubit® instrument layout:

**Figure 1: The Qubit® 3.0 Fluorometer**



1. **REAGENTS:**
   1. Invitrogen dsDNA Quantification, Broad Range Kit (Fisher Scientific, Cat#Q32850).
      1. dsDNA Broad Range Buffer, store at room temperature.
      2. dsDNA BR Fluorescent dye, store at room temperature away from light.
      3. dsDNA Broad Range Standards, store at 2-8˚C.
   2. Invitrogen dsDNA Quantification, High Sensitivity Kit (Fisher Scientific, Cat#Q32851).
      1. dsDNA High Sensitivity Buffer, store at room temperature.
      2. dsDNA HS Fluorescent dye, store at room temperature away from light.
      3. dsDNA High Sensitivity Standards, store at 2-8˚C.
   3. Invitrogen RNA Quantification, Broad Range Kit (Fisher Scientific, Cat#Q10210).
      1. RNA Broad Range Buffer, store at room temperature.
      2. RNA BR Fluorescent dye, store at room temperature away from light.
      3. RNA Broad Range Standards, store at 2-8˚C.
   4. Invitrogen RNA Quantification, High Sensitivity Kit (Fisher Scientific, Cat#Q32852).
      1. RNA High Sensitivity Buffer, store at room temperature.
      2. RNA HS Fluorescent dye, store at room temperature away from light.
      3. RNA High Sensitivity Standards, store at 2-8˚C.
2. **PROCEDURE FOR OPERATION:**
   1. Starting the Qubit® 3.0 Fluorometer:
      1. Plug one end of the supplied power cord into the power inlet of the instrument.
      2. Attach the appropriate plug adaptor to the other end of the power cord.
      3. Plug the power cord into the electrical outlet.
      4. The instrument automatically powers on, first displaying the splash screen, and then the Home Screen.
   2. Shutting down the Qubit® 3.0 Fluorometer:
      1. To power down the instrument, unplug it from the power source.
   3. Operating the Qubit® 3.0 Fluorometer:
      1. On the Home Screen, the user has the option to:
         1. Select a protocol from the list of Qubit® quantitation assays – **dsDNA**, **RNA**, **oligo** (**ssDNA**), **protein**.
         2. Access quantitation data – data can be exported for documentation.
         3. Configure instrument settings – functions such as sleep mode, brightness of the display, date/time, instrument reset, and language preference can be configured from this option.
      2. During operation of the Qubit® 3.0 Fluorometer, the following guidelines must be followed:
         1. Only thin-wall, clear 0.5-mL PCR tubes are appropriate for use in the Qubit® 3.0 Fluorometer (**Qubit® assay tubes Cat. no. Q32856 or Axygen PCR-05-C tubes part no. 10011-830**).
         2. Wear gloves during sample handling.
         3. Use the instrument at room temperature only (22-28°C).
         4. Bring all kit reagents to room temperature prior to use.
         5. Insert assay tube into the instrument only for as much time as it takes for the instrument to measure the fluorescence.
         6. Do not hold the assay tubes in your hand before performing a measurement (added heat may affect the accuracy of the reading).
         7. Make sure that the desired Qubit® assays are calibrated using the appropriate standards.
         8. If you are performing multiple readings of a single tube, remove the tube from the instrument and let it equilibrate to room temperature for 30 seconds before taking another reading.
      3. A calibration must be performed and passed for each Qubit® assay prior to running the assay. Assay calibrations must also be performed per QC/maintenance requirement.
      4. The Qubit® uses a two-point **Standard** calibration process for nucleic acids and a three-point **Standard** calibration process for proteins.
   4. Preparing a Qubit® Assay Reactions
      1. Standard and Sample reactions must be prepared prior to running the Qubit®. For best results, ensure that the reagents are at room temperature before performing a measurement.
      2. Begin by preparing the Qubit® Working Solution, which is a 1:200 dilution of Qubit® reagent and Qubit® buffer:
         1. For each reaction (Standard or Sample), combine 1µL of the desired Qubit® reagent with 199 µL of the corresponding Qubit® buffer.
         2. Prepare adequate amount of Working Solution for each measurement. Do **not** vortex the working solution before the addition of DNA or RNA, pipette mix ONLY.
         3. See Table 2 for the Qubit® Working Solution Preparation Guide:

**Table 2: Qubit® Working Solution Preparation**

|  |  |  |
| --- | --- | --- |
| Number of Reactions (Sample and/or Standards) | Volume of Qubit® Buffer (µL) | Volume of Qubit® Reagent (µL) |
| 1 | 398 | 2 |
| 2 | 597 | 3 |
| 3 | 796 | 4 |
| 4 | 995 | 5 |
| 5 | 1194 | 6 |
| 6 | 1393 | 7 |
| 7 | 1592 | 8 |
| 8 | 1791 | 9 |
| 9 | 1990 | 10 |
| 10 | 2189 | 11 |
| 11 | 2388 | 12 |
| 12 | 2587 | 13 |
| 13 | 2786 | 14 |
| 14 | 2985 | 15 |
| 15 | 3184 | 16 |
| 16 | 3383 | 17 |

* 1. Performing a Qubit® Assay Reading:
     1. Performing the Calibration:
        1. **NOTE:** Calibration should be run monthly and when a new kit/lot is opened.
        2. Set up two assay tubes for nucleic acid **Standards,** accordingly:
        3. To each assay tube, add 190 µL of the previously made Working Solution.
        4. Add 10 µL of the appropriate **Standard**; total volume should be 200 µL.
        5. Vortex all tubes for 2-3 seconds.
        6. Incubate the assay tubes for 2 minutes at room temperature, away from light.
        7. On the Home Screen, touch the desired assay type to calibrate (e.g., dsDNA, RNA, Oligo).
        8. Next, select the quantitation range of the assay type previously selected to calibrate (e.g., dsDNA: High sensitivity, dsDNA: Broad range, RNA: High Sensitivity, and RNA: Broad Range).
        9. **dsDNA High Sensitivity (dsDNA HS)** – for DNA sample concentration (0.01ng/µL – 100 ng/ µL) (standard procedure for DNA quantitation).
        10. **dsDNA Broad Range (dsDNA BR)** – for DNA sample concentration (0.1ng/µL – 1000 ng/ µL).
        11. **RNA High Sensitivity (RNA HS)** – for RNA sample concentration (0.25ng/µL – 100 ng/ µL) (standard procedure).
        12. **RNA Broad Range (RNA BR)** – for RNA sample concentration (1ng/µL – 1000 ng/ µL).
        13. If the selected assay has already been calibrated, the user will be prompted to choose between reading “New Standards” or “Running Samples”.
        14. Select “Run Samples” if the calibration for that assay is up to date. Otherwise, select “New Standards” to perform a calibration for that assay.
        15. On the “Sample volume screen”, select the **sample volume** (**2 µL**) and **units** (**ng/µL**). See Figure 5.
            1. Touch the “+” or “–“ buttons on the wheel to change the sample volume added to the assay tube.
            2. From the dropdown menu, select the units for the output sample concentration.
        16. When prompted, insert **Standard #1** into the sample chamber and touch **Read standard.**
        17. When prompted, insert **Standard #2** into the sample chamber and touch **Read standard.**

**NOTE:** Be sure to use the correct Standards. The reading takes approximately 3 seconds.

* + - 1. The calibration is complete after **Standard #2** is read.
      2. The software will display the results:
         1. If the calibration is successful, the “Read Standard screen” will show a *Fluorescence vs. Concentration graph* (see Figure 2).
         2. If the calibration is not successful, the software displays the “Calibration error” message (see Figure 3). The user may choose to re-run the **Standards** or prepare and run new **Standards**.

**NOTE:** For the Qubit® Nucleic Acid assays, the reading given by **Standard #2** should be **at least ten times higher** than that of **Standard #1**.

* + 1. Performing a Sample reading:
       1. Prepare the assay-specific Qubit® Working Solution by combining the appropriate volumes of Qubit® reagent and buffer. See Table 2.
       2. In each assay tube, add the 198µL of Working Solution.
       3. Then, add 2µL of the appropriate **Sample** for a total volume of 200µL (see Table 3).
       4. Vortex the assay tubes for 2-3 seconds.
       5. Incubate the tubes for 2 minutes at room temperature away from light.

**Table 3: Reaction Tube Preparation (Samples)**

|  |  |
| --- | --- |
| Volume of Working solution | 198 µL |
| Volume of Sample to add | 2 µL |
| Total volume in each Assay Tube | 200 µL |

* + - 1. Using a clean paper towel, wipe each assay tube before inserting into the Sample Chamber to prevent inaccurate reading due to residue on the tube.
      2. After incubation, insert the assay tube into the sample chamber, close the lid, and then touch **Read tube**. The reading takes approximately 3 seconds. The software displays the results on the “Results screen”. See Figure 6.
      3. If the results are within the assay’s range, the concentration values are displayed as:
         1. The top value (in large font) is the concentration of the original sample.
         2. The bottom value is the dilution concentration (the concentration of the sample in the tube inserted into the Qubit® Fluorometer).
      4. If the results are outside of the assay’s range, an “Out of Range” message is displayed. See Figure 7.
      5. For samples that are “Out of Range”, perform the following:
         1. “Too High” – use the Qubit® RNA BR Assay (Broad-Range) for samples with concentrations above the range of the Qubit® RNA HS Assay (High-Sensitivity).
         2. “Too Low” – for samples with concentrations below the range of the Qubit® Assay, request additional specimen with sufficient tissue/cellularity.
      6. To read multiple samples for the same assay:
         1. Remove the current assay tube and insert a new assay tube.
         2. Touch **Read tube**.
    1. To view the Fluorescence vs. Concentration graph, **swipe left** or touch the **right arrow** (see Figures 8-11):
       1. **Open circles** – represent correct standards.
       2. **The large gray circle** – represents the most recent sample.
       3. **Blue circles** – represent samples that fall within the assay’s core range.
       4. **Yellow circles** – represent samples that fall within the assay’s extended range.
       5. **Red circles/dot** – represent samples or **Standards** that fall outside the assay’s range.

**NOTE:** The Qubit® 3.0 Fluorometer automatically stores numeric data from all reads but does not store graphic data.

* 1. Managing Data:
     1. The Qubit® 3.0 Fluorometer can save data for up to 1000 samples and allows the user to:
        1. View detailed data for each sample.
        2. Rename data files.
        3. Save data as a .CSV file and export the .CSV file to a USB drive or directly to a computer.
        4. Delete data files.
     2. **To view detailed sample data**:
        1. From the **Concentration**, **Graph**, or **Home** screens, touch **Data**.
        2. On the “Export data screen”, touch the data set of interest to display a list of data entries for that data set.
        3. Scroll up or down to view additional data entries that do not fit in the screen.
        4. Touch the sample of interest to view the sample details.
        5. To name the Sample IDs export the data and edit the Excel file on a Lifespan computer.
     3. **To export data**:
        1. Re-insert the USB drive into the Qubit® 3.0 Fluorometer, if the Qubit drive is not found on the computer.
        2. From the **Concentration**, **Graph**, or **Home** screens, touch **Data**.
        3. Select **Export**.
        4. On the “Export data screen”, check the selection box to the left of each data set you wish to export.
        5. The user can choose to export all data as an Excel file to the Qubit folder, which is located on the Lifespan computer connected to the instrument.
        6. To save only individual data entries from a data set, touch the data set of interest, and then check the selection box to the left of the sample(s) you wish to export.
        7. Touch **Export** to export the data.

**NOTE**: The numeric data is automatically saved as a .CSV file.

* + 1. **Once the file has been exported:**
       1. On the computer connected to the Qubit, navigate to the Qubit drive.
       2. Open the Qubit **Internal Storage** folder.
       3. Open the **Qubit3 folder.**
       4. Open the folder containing your data.
       5. Name the file using the format: [YYYYMMDD\_NucleicAcid\_Qubit\_Readings]
          1. Ex. 20200908\_RNA\_Qubit\_Readings
       6. Move the document by cutting and pasting from the Qubit folder to the MGP\_Qubit Import Files folder in the RICMBLAB$ shared drive.
       7. Open the Qubit Reading document.
       8. Edit the Test Name column to reflect the Sample ID’s of samples read. Scan the sample tubes to rename the Test Name for each sample.

**NOTE**: Remember when Qubit reading document is exported, the samples are exported with the first read tube last and the last read tube first.

* + - 1. Select **Save As**.
      2. Change the file type to **Excel Workbook (\*.xlsx)**.
      3. Select **Save**.
    1. **Dilution Calculations:**
       1. **Soft Molecular will automatically calculate dilutions to 100 ng/ul.**
       2. To dilute TNA to a final concentration of 100 ng/ul:
          1. Use formula: (C1)(V1)=(C2)(V2)
          2. C1 = concentration read from spectrophotometer
          3. V1 = volume in tube from extraction (typically 35ul per tube on Maxwell, 100ul organic extraction)
          4. C2 = 50ng/ul or 100ng/ul
          5. Solve for V2, which is the total volume
          6. To get final desired concentration, volume to add = V2 -V1.
    2. **To delete data files**:
       1. Touch **Data** from the **Concentration**, **Graph**, or **Home** screens.
       2. On the “Export data screen”, check the selection box to the left of each data set you wish to delete.
       3. To delete only individual data entries from a data set, touch the data set of interest, and then check the selection box to the left of the sample(s) you wish to delete.
       4. Touch **Delete** to **permanently** delete the sample data or data set.
       5. Touch **Cancel** to return to the screen previously viewed without deleting any data.

1. **QC AND MAINTENANCE:** 
   1. The Qubit® 3.0 Fluorometer does not need regular maintenance. Periodic cleaning is recommended to prevent the buildup of dust and dirt that might reduce the instrument’s performance and cause contamination. Always unplug the fluorometer before cleaning.
   2. **Monthly**:
      1. Clean the surface of the Qubit® 3.0 Fluorometer by gently wiping with a Glass/LCD anti-static wipe. (Glass/LCD Wipes from Grainger, Cat. # 6MGG8)
      2. Follow immediately by wiping with a clean/dry Kimwipe for a streak-free clean.
      3. Perform Qubit kit calibration monthly or when a new kit/lot is opened.
         1. Record calibration standard results on the Qubit 3.0 Assay Calibration Form.
            1. File path: RICMBLAB$ network drive\QC\Qubit 3.0 folder.
   3. **As Needed:**
      1. To disinfect the instrument, disconnect the power cable from the Qubit® 3.0 Fluorometer and clean the instrument, including the touch screen, with a soft cloth/wipe lightly moistened with 70% ETOH, 70% isopropanol, or 10% bleach. Repeat with a Glass/LCD wipe, followed by a clean/dry Kimwipe.

**NOTE**: To prevent the touch screen from getting scratched, do not use abrasive cleaning solutions or materials.

**NOTE**: Ensure that the cleaning solution does not enter the power button, the power inlet, the sample port, or the USB drive ports.

**NOTE**: To avoid electrical shock when the instrument is plugged in, never pour, or spray any liquids directly on the instrument.

* + 1. To update the Qubit® 3.0 Fluorometer with the latest software from Life Technologies, refer to pages 48-49, **Software update**, of the Qubit® 3.0 Fluorometer User Guide. Updates should only be performed with Director Approval and appropriately documented.
    2. Perform the device verification test when a problem with the instrument is suspected (refer to page 50, **Device verification test**, of the Qubit® 3.0 Fluorometer User Guide).

1. **TROUBLESHOOTING:**
   * 1. For troubleshooting, refer to Appendix A: Troubleshooting of the Qubit® 3.0 Fluorometer User Guide.
2. **CONTACT INFORMATION:**
   1. Life Technologies Corporation

USA

Website: www.lifetechnologies.com/Qubit®

E-mail: techsupport@lifetech.com

Toll Free: 1-800-955-6288 x45682

1. **REFERENCES:**
   1. Qubit® 3.0 Fluorometer User Guide.
   2. Qubit® 3.0 Fluorometer Quick Reference Qubit® assays.
2. **ATTACHMENTS:**
   1. Qubit 3.0 Fluorometer Instrument Maintenance Form
   2. Qubit 3.0 Assay Calibration Form
3. **REVISIONS:**
   1. 12/14/2020: Updates were made to the preparing assay reactions, performing assay readings and saving of files.
   2. 2/23/2023: Update maintenance section to reflect monthly instrument calibration.