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MyQubit Amplex® Red Glucose Assay

Introduction

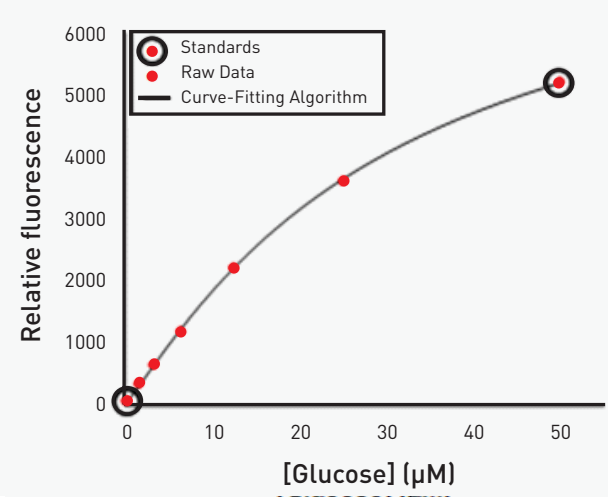
The MyQubit Amplex® Red Glucose Assay for use with the Qubit® 2.0 Fluorometer, which is available for downloading from www.lifetechnologies.com, allows easy and accurate quantification of glucose using the Amplex® Red Glucose/Glucose Oxidase Assay Kit (Cat. no. A22189) and the Amplex® Red/UltraRed Stop Reagent (Cat. no. A33855). The assay provides a sensitive one-step method for detecting glucose and can be very useful for the quantification of glucose levels in foods, fermentation media, and bodily fluids. The MyQubit Amplex® Red Glucose Assay file can be downloaded from our website and permanently uploaded to your Qubit® 2.0 Fluorometer following the guidelines described below.

Amplex® Red reagent (10-acetyl-3,7-dihydroxyphenoxazine) is a colorless, stable, and extremely versatile peroxidase substrate. Because peroxidase- and glucose oxidase-mediated reactions can be coupled,[1,2] it is possible to measure the release of glucose by any glucosidase enzyme (e.g., β-glucosidase). In the assay, glucose oxidase reacts with D-glucose to form D-gluconolactone and H_2O_2. In the presence of horseradish peroxidase (HRP), Amplex® Red reagent reacts with H_2O_2 with in a 1:1 stoichiometry to produce highly fluorescent resorufin.[1,2] Because resorufin has a fluorescence emission maximum of approximately 585 nm, there is little interference from autofluorescence in most biological samples.

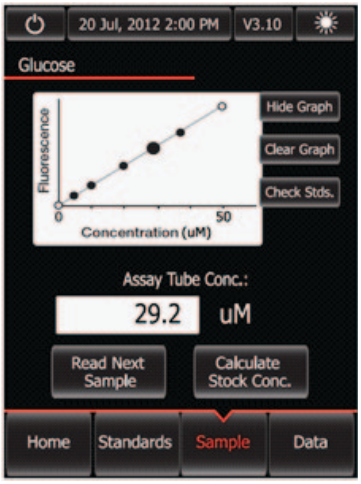
Using the MyQubit Amplex® Red Glucose assay, we have detected as little as 250 nM glucose in the assay tube (Figure 1).

Figure 1 (A) The plot showing the line corresponding to the curve-fitting algorithm (a Modified Hill plot) used to calculate concentration in the MyQubit Amplex® Red Glucose Assay. For reference, the positions of the standards and a set of data points from an actual experiment are shown superimposed onto the line, demonstrating that the curve-fitting algorithm gives accurate values for quantitation. **(B)** The assay has the same look and feel of the existing Qubit® assays.

A



B



In addition to the MyQubit Amplex® Red Glucose Assay for quantifying cholesterol, the MyQubit firmware allows you to create other assays for your Qubit® 2.0 Fluorometer. All new Qubit® 2.0 instruments are pre-loaded with the MyQubit firmware, which can also be downloaded from www.lifetechnologies.com/qubit for installation into existing instruments. Using the MyQubit firmware, additional assays can be created based on existing Life Technologies reagents and assays or completely novel ideas. Since the instrument is operated by simple commands, creating additional applications can be as straightforward as matching spectral compatibility with the LEDs and emission filters. Detailed instructions and templates for creating new MyQubit assays are also provided on the Qubit® website (www.lifetechnologies.com/qubit).

Before You Begin

Firmware Requirements

Your Qubit® 2.0 Fluorometer must have V3.10 firmware or later installed for you to be able to upload new assays using the MyQubit function. Before proceeding, make sure that your Qubit® 2.0 Fluorometer has been upgraded to V3.10 firmware or later.

Materials Required

- Amplex® Red Glucose/Glucose Oxidase Assay Kit (Cat. no. A22189)*
- Amplex® Red/UltraRed Stop Reagent (Cat. no. A33855)**
- Qubit® 2.0 Fluorometer (Cat. no. Q32866)
- MyQubit Amplex® Red Glucose assay file (Amplex Glucose.qbt), available for downloading at www.lifetechnologies.com/qubit
- USB drive clear of other .qbt files
- Qubit® Assay Tubes (Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR part no. 10011-830)
- Ethanol and E-pure H_2O for reconstituting Amplex® Red/UltraRed Stop Reagent
- Plastic tubes for preparing buffers and dilutions of standards/samples.

* Each Amplex® Red Glucose/Glucose Oxidase Assay Kit provides sufficient reagents for 250 assays using the Qubit® 2.0 Fluorometer and the protocol described below.

** Each vial of Amplex® Red/UltraRed Stop Reagent provides sufficient reagent to terminate 50 assays using the Qubit® 2.0 Fluorometer and the protocol described below (Cat. no. A33855 includes 5 vials).

Downloading the .qbt File from the Web

Download the MyQubit Amplex® Red Glucose assay file (Amplex Glucose.qbt) from www.lifetechnologies.com/qubit and save it directly to your PC. Then, transfer the file from your computer to the root directory of your USB drive. Ensure that you only have a single .qbt file on your USB drive before uploading it to the Qubit® 2.0 Fluorometer.

Note: Downloading a .qbt file from the web directly to your USB drive may result in unexpected behavior.

Uploading the .qbt File to the Qubit® 2.0 Fluorometer

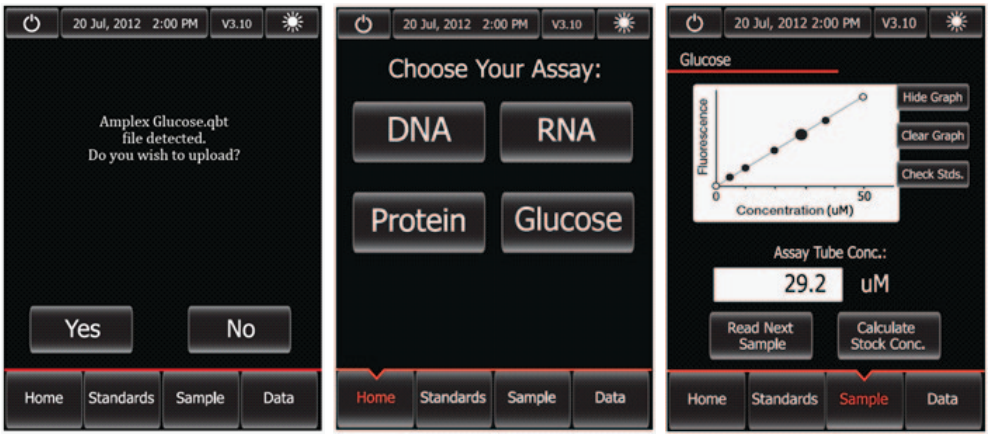
1.1 Make sure there is only one .qbt file on your USB drive.

1.2 With your Qubit® 2.0 Fluorometer unplugged, insert the USB drive containing the MyQubit Amplex® Red Glucose assay file (Amplex Glucose.qbt) into the USB port on the instrument.

1.3 Plug the Qubit® 2.0 Fluorometer back in to power it on. The instrument will display the following message: "Amplex Glucose.qbt file detected. Do you wish to upload?" Click **Yes** to proceed with the upload, which will take ~2 seconds.

1.4 Once the upload is complete, you will be directed to a new Home Screen displaying a new button called "Glucose", which indicates that the cholesterol assay is permanently uploaded to the instrument (Figure 2). You do not need the USB drive to access the assay. Functionality of the pre-existing assays is not affected in any way.

Figure 2 Upload of the MyQubit Amplex® Red Glucose assay. The Qubit® 2.0 Fluorometer will recognize the Amplex Glucose.qbt file and guide you through the upload process.



Material Storage and Handling

- Upon receipt, the Amplex® Red Glucose/Glucose Oxidase Assay Kit should be stored frozen at ≤ –20°C, protected from light. When stored properly, the kit components are stable for at least six months.
- Allow the reagents to warm to room temperature before opening the vials.
- The Amplex® Red reagent is somewhat air sensitive. Once a vial of Amplex® Red reagent is opened, the reagent should be used promptly.
- Protect the Amplex® Red reagent from light.
- Store Amplex® Red/UltraRed Stop Reagent refrigerated at ≤ 2–8°C until required for use. If frozen, avoid freeze-thaw cycles. Desiccation is recommended but not essential.
- Refer to the detailed product information provided with the materials for additional storage and handling information, including disposal.

Critical Assay Parameters

Incubation Time

The MyQubit Amplex® Red Glucose assay for use with the Qubit® 2.0 Fluorometer was optimized using Amplex® Red/UltraRed Stop Reagent (Cat. no. A33855). This reagent provides a means to terminate the fluorescence signal-generating reaction in the enzymatic assay. Once the stop reagent has been added (after 15 minutes), fluorescence signal remains stable for at least 3 hours (i.e., sample reads remain within 10% average deviation from the expected value using the same standard calibration). This enables the reading of multiple user samples during this time frame without requiring a new calibration. Samples that are read more than 3 hours after calibration may exhibit increased deviation from the actual concentration values.

Preparing the Amplex® Red/ UltraRed Stop Reagent

Reconstitute one vial of Amplex® Red/UltraRed Stop Reagent by adding 1.45 mL of ethanol and vortex or agitate briefly. Transfer 1.25 mL of this solution to a vial and dilute with 1.25 mL of E-pure H_2O, giving a total of 2.5 mL of stop solution. This amount is sufficient to stop 50 assays of 200 µL each, based on the protocol described below. After reconstitution, the stop reagent is stable for approximately one month when stored at 2–8°C, protected from light. The appearance of amber coloration is indicative of decomposition.

Calibrating the Qubit® 2.0 Fluorometer

For each assay, you have the choice to run a new calibration or to use the values from the previous calibration. As you first start using the instrument, perform a new calibration each time. As you become familiar with the assays, the instrument, your pipetting accuracy, and significant temperature fluctuations within your laboratory, determine the level of comfort you have using the calibration data stored from the last time the assay was calibrated. Remember that after the addition of the Amplex® Red/ UltraRed Stop Reagent, the fluorescence signal in the tubes containing standards and the samples is stable for at least 3 hours when stored at room temperature. For best results, perform a new calibration each time a new working solution is prepared.

Calculating the Concentration of Your Sample

The Qubit® 2.0 Fluorometer gives values for the MyQubit Amplex® Red Glucose assay in µM. This value corresponds to the concentration after your sample was diluted into the assay tube. To calculate the concentration of your starting sample, use the following equation:

$$\text{Concentration of your sample} = \text{QF value} \times \frac{240}{x}$$

where QF value is the value given by the Qubit® 2.0 Fluorometer, and x is the volume of sample in microliters added to the assay tube.

This equation generates a result with the same units as the value given by the Qubit® 2.0 Fluorometer in the assay tube. Because the MyQubit Amplex® Red Glucose Assay gives concentrations in µM, the result of the equation above will be in µM.

Dilution Calculator

The Dilution Calculator function will not be useful when using the MyQubit Amplex® Red Glucose Assay on the Qubit® 2.0 Fluorometer. The Dilution Calculator function was designed to accommodate sample dilutions of 1–20 µL in a final assay volume of 200 µL, based on the protocols for the existing assays for DNA, RNA, and protein quantification; it is therefore not relevant for the dilution schemes used here.

Experimental Procedure

Preparing Buffers and Reagents

Prepare all kit reagents (i.e., buffers, stock and working solutions) according to the instructions provided with the Amplex® Red Glucose/Glucose Oxidase Assay Kit.

Performing the MyQubit Amplex® Red Glucose Assay

The protocol below describes the MyQubit Amplex® Red Glucose assay in a total volume of 240 µL per microplate well including the Amplex® Red/UltraRed Stop Reagent (see Table 1). The final concentrations of assay standards and samples have been adjusted for the additional volume based on the dilution scheme outlined in the protocol below. The volumes recommended here are sufficient for 250 assays.

Table 1 Volume of reagents used in the MyQubit Amplex® Red Glucose assay

	Standard Assay Tube	User Sample Assay Tube
Volume of **Working Solution** (from Step 2.4)	100 µL	100 µL
Volume of **Standard**	100 µL	—
Volume of **User Sample**	—	100 µL
Volume of **Stop Reagent**	40 µL	40 µL
Total volume in each Assay Tube	240 µL	240 µL

Note: The instructions provided with the Amplex® Red Glucose/Glucose Oxidase Assay Kit may be used for reagent preparation. However, given the difference in reagent volumes used in the MyQubit cholesterol assay and the assay kit, the following procedure should be used in place of the Glucose Assay protocol (Section 2) provided with the Amplex® Red Glucose/Glucose Oxidase Assay Kit.

Refer to the Qubit® 2.0 Fluorometer User Guide, available for for downloading at www.lifetechnologies.com/qubit, for detailed instructions on instrument use.

2.1 Set up two Assay Tubes for the standards and one for each user sample. Use only thin-walled, clear 0.5 mL PCR tubes. Acceptable tubes include Qubit® assay tubes (set of 500, Cat. no. Q32856) or Axygen PCR-05-C tubes (VWR, part no. 10011-830).

2.2 Prepare a dilution of the glucose reference standard for use as Standard 2 in calibration of the Qubit® assay by adding 30 µL of the 400 mM glucose reference standard (prepared according to instruction provided with the Amplex® Red Glucose/Glucose Oxidase Assay Kit) to 970 µL of 1X Reaction Buffer. This 1 mL of 120 µM glucose solution is sufficient to perform 10 standard calibrations. Store any unused portion of standard at < –20°C.

Note: MyQubit Amplex® Red Glucose assay calibration requires a final concentration of 50 µM glucose in the assay well. Since the glucose concentration will be 2.4-fold lower in the final reaction volume (after addition of the Amplex® Red/UltraRed Stop Reagent), the glucose reference standard is diluted to 120 µM in 1X Reaction Buffer.

2.3 Prepare calibration standards by adding 100 µL of 1X Reaction Buffer to one assay tube (Standard 1) and 100 µL of 120 µM glucose solution to another assay tube (Standard 2).

2.3 Dilute your glucose-containing samples in 1X Reaction buffer. A volume of 100 µL will be used for each reaction. You may wish to prepare several different dilutions for each sample. Add 100 µL of each sample to a separate assay tube.

2.4 Prepare a working solution of 100 µM Amplex® Red reagent containing 0.2 U/mL HRP and 2 U/mL glucose oxidase by adding 10 µL of Amplex® Red reagent stock solution, 20 µL of HRP stock solution, and 20 µL of glucose oxidase stock solution to 0.95 mL of 1X Reaction Buffer.

2.5 Start the reactions by adding 100 µL of the Amplex® Red reagent/HRP/glucose oxidase working solution to each assay tube containing standard and sample. Try to add the working solution to all assay tubes at the same time; if possible, use a multi-channel or repeat pipettor.

2.6 While the standard and sample reactions are incubating at room temperature, protected from light, reconstitute the Amplex® Red/UltraRed Stop Reagent according to the instructions provided product (also described above under "Critical Assay Parameters", page 4).

2.7 After incubating the reactions for 15 minutes at room temperature, protected from light, add 40 µL of prepared Amplex® Red/UltraRed Stop Reagent to each assay tube, standards and sample(s).

2.8 Vortex all of the tubes for 2–3 seconds.

2.9 Calibrate the assay and read samples by selecting "Glucose" from the Home Screen and following the on-screen instructions, similar to the operation of the existing assays. For detailed instructions, refer to the Qubit® 2.0 Fluorometer user guide, available for downloading at www.lifetechnologies.com/qubit. The concentration reported by the instrument is the concentration of glucose in the actual assay tube.

Note: The Dilution Calculator function will not be useful when using the MyQubit Amplex® Red Glucose Assay on the Qubit® 2.0 Fluorometer. The Dilution Calculator function was designed to accommodate sample dilutions of 1–20 µL in a final assay volume of 200 µL, based on the protocols for the existing assays for DNA, RNA, and protein quantification; it is therefore not relevant for the dilution schemes used here. To calculate the concentration of your starting sample, use the following formula:

$$\text{Concentration of your sample} = \text{QF value} \times \frac{240}{x}$$

where QF value is the value given by the Qubit® 2.0 Fluorometer, and x is the volume of sample in microliters added to the assay tube.

Appendix

1. You may omit the use of the Amplex® Red/UltraRed Stop Reagent, if desired. Doing so allows the enzymatic reaction to continue longer, which may be useful for samples containing low concentrations of glucose. Samples such as this may require longer incubation times to generate fluorescence signal sufficient to provide reliable quantification. However, the parameters used in the .qbt file for the MyQubit Amplex® Red Glucose assay have not been validated in the absence of Amplex® Red/UltraRed Stop Reagent. The shape of the standard curve used in calibration will be altered with variations in incubation time. In addition, since the Qubit® 2.0 Fluorometer is designed for low-to-mid throughput use and is equipped to read only a single sample at a time, variation in incubation time could potentially result in diminished accuracy. Therefore, we recommend customizing the parameters in the Amplex Glucose.qbt file to optimally suit your needs. For detailed instructions on how to create a custom assay using MyQubit, refer to www.lifetechnologies.com/qubit.

   Conversely, you may use Qubit® Raw mode to collect raw fluorescence data over multiple time points to measure the kinetics of the reaction.

2. The Amplex® Red Glucose/Glucose Oxidase Assay Kit has also been shown to be compatible with Amplex® UltraRed reagent. The Amplex® UltraRed reagent (Cat. no. A36006) provides all of the same performance characteristic of Amplex® Red while displaying improved stability over a larger pH range and, in some cases, increased sensitivity. The same assay protocol and reagent dilutions may be used with Amplex® UltraRed reagent. However, for best results, we recommend optimizing the parameters in the Amplex Glucose.qbt file for use with Amplex® UltraRed reagent. For detailed instructions on how to create a custom assay using MyQubit, refer to www.lifetechnologies.com/qubit.

References

1. Proc SPIE-Intl Soc Opt Eng 3606, 119 (1999); 2. Anal Biochem 253, 162 (1997); 3. J Immunol Methods 202, 133 (1997).

Product List

Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
Q32866	Qubit® 2.0 Fluorometer	each
A22189	Amplex® Red Glucose/Glucose Oxidase Assay Kit	1 kit
A33855	Amplex® Red/UltraRed Stop Reagent	1 set
Q32856	Qubit® Assay Tubes	1 set

Purchaser Notification

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Additional international offices are listed at www.lifetechnologies.com

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

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