invitrogen
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MyQubit Amplex™ Red Sucrose Assay

Introduction

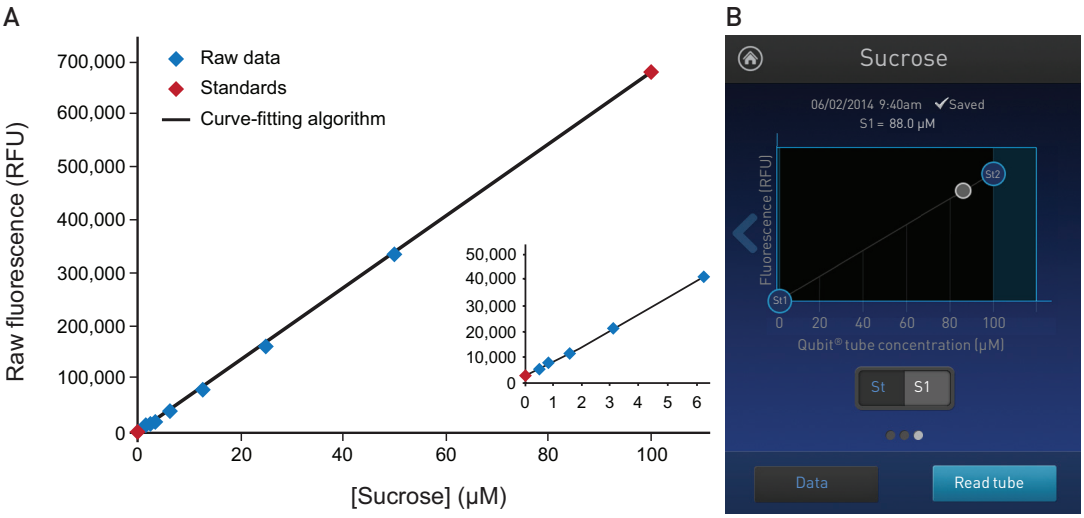
The MyQubit Amplex™ Red Sucrose Assay for use with the Qubit™ Fluorometer (available for download from **www.lifetechnologies.com**) allows easy and accurate quantification of sucrose using the Amplex™ Red Glucose/Glucose Oxidase Assay Kit (Cat. no. A22189), invertase (MP Biomedicals, Cat. no. 0215134550), and the Amplex™ Red/UltraRed Stop Reagent (Cat. no. A33855). The assay provides a sensitive method for detecting sucrose and can be used for the quantification of sucrose levels in foods, bodily fluids, and fermentation products such as wine or beer. The MyQubit Amplex™ Red Sucrose Assay enables the Qubit™ Fluorometer to calculate and display the sucrose concentration of samples based on an optimized standard curve algorithm.

The Amplex™ Red reagent (10-acetyl-3,7-dihydroxyphenoxazine) is a colorless, stable, and extremely versatile peroxidase substrate. In the assay, invertase hydrolyzes sucrose into D-glucose and D-fructose. Glucose oxidase then reacts with D-glucose to form D-gluconolactone and hydrogen peroxide (H_2O_2). In the presence of horseradish peroxidase (HRP), Amplex™ Red reagent reacts with H_2O_2 in a 1:1 stoichiometry to produce highly fluorescent resorufin.[1,2] This series of reactions allows for the quantification of sucrose based on resorufin fluorescence. 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 Sucrose assay, we have detected as little as 500 nM sucrose in the assay tube (Figure 1, page 2). Please note that the MyQubit Amplex™ Red Sucrose assay will also detect D-glucose. If glucose contamination is suspected in the sample, we suggest first performing the MyQubit Amplex™ Red Glucose assay. If the concentration of glucose is significant, you will then need to run a glucose control for each sample analyzed by the MyQubit Amplex™ Red Sucrose assay.

Note that the product of the Amplex™ Red reaction is unstable in the presence of thiols such as dithiothreitol (DTT) or 2-mercaptoethanol. For this reason, the final DTT or 2-mercaptoethanol concentration in the reaction should be less than 10 µM. The absorption and fluorescence of resorufin are pH-dependent. Below the pK_a (~6.0), the absorption maximum shifts to ~480 nm and the fluorescence quantum yield is markedly lower. In addition, the Amplex™ Red reagent is unstable at high pH (>8.5). For these reasons, the reaction should be performed at pH 7–8, for example by using the provided reaction buffer (pH 7.5).

Figure 1 (A) The plot showing the line corresponding to the curve-fitting algorithm (a Modified Hill plot) used to calculate galactose concentration in the MyQubit Amplex™ Red Sucrose Assay. For reference, the positions of the standards (in red) 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 as the existing Qubit™ assays.



In addition to using the existing MyQubit Assays, users can create new assays for the Qubit™ Fluorometer using the MyQubit firmware. Such assays can be created based on existing Thermo Fisher Scientific 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 requirements for the proposed assay with those offered by the Qubit™ Fluorometer. The Qubit™ 3.0 Fluorometer and newer Qubit™ 2.0 instruments are pre-loaded with the MyQubit firmware. For older Qubit™ 2.0 instruments, a firmware update can be downloaded from **www.lifetechnologies.com/qubit**.

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 (Qubit™ 2.0 only)

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.

No firmware updates are required for Qubit™ 3.0 instruments.

Materials required

- Amplex™ Red Glucose/Glucose Oxidase Assay Kit (Cat. no. A22189)*
- Invertase (100 U/mg) (MP Biomedicals, Cat. no. 0215134550)
- Sucrose (Fisher, Cat. no. BP220)
- Amplex™ Red/UltraRed Stop Reagent (Cat. no. A33855)**
- Qubit™ Fluorometer (Cat. no. Q33216)
- MyQubit Amplex™ Red Sucrose assay file (Amplex Sucrose.qbt), available for downloading at **www.lifetechnologies.com/qubit**.

  Note: Different files are required for Qubit™ 2.0 and 3.0 instruments.
- USB drive clear of other .qbt files ***
- Qubit™ Assay Tubes (Cat. no. Q32856) or Axygen PCR-05-C tubes (Thermo Fisher Scientific, Cat. no. 14-222-292)
- Ethanol and E-pure H_2O
- Plastic tubes for preparing buffers and dilutions of standards and samples

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

** Each vial of Amplex™ Red/UltraRed Stop Reagent (Cat. no. A33855, includes 5 vials) provides sufficient reagents to terminate 35 assays using the Qubit™ Fluorometer and the protocol described below.

*** For Qubit™ 3.0 instruments, the USB drive may contain multiple .qbt files.

Material storage and handling

- Upon receipt, store the Amplex™ Red Glucose/Glucose Oxidase Assay Kit and invertase 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, use the reagent promptly.
- Protect the Amplex™ Red reagent from light.
- Store the 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.

Download the .qbt file from the web

Download the MyQubit Amplex™ Red Sucrose Assay file (Amplex Sucrose.qbt) from **www.lifetechnologies.com/qubit** and save it directly to your computer. Be sure to download the appropriate file specific to your Qubit™ instrument, either the 2.0 file or the 3.0 file. 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 (for Qubit™ 3.0 instruments, the USB drive may contain multiple .qbt files).

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

Upload the .qbt file to the Qubit™ 2.0 Fluorometer

1.1 Load the Amplex™ Red Sucrose Assay file (Amplex Sucrose.qbt) onto your USB drive. The firmware only allows for one assay to be uploaded at a time; therefore, the USB drive should contain only one .qbt file.

1.2 With your Qubit™ 2.0 Fluorometer unplugged, insert the USB drive containing the MyQubit Amplex™ Red Sucrose Assay file (Amplex Sucrose.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 Sucrose.qbt file detected. Do you wish to upload?" Select **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 "Sucrose", which indicates that the Sucrose Assay is permanently uploaded to the instrument. You do not need the USB drive to access the assay. Functionality of the pre-existing assays is not affected in any way.

Upload the .qbt file to the Qubit™ 3.0 Fluorometer

2.1 Load the Amplex™ Red Sucrose Assay file (Amplex Sucrose.qbt) onto your USB drive. The USB drive may contain more than one .qbt file.

2.2 Insert the USB drive containing the MyQubit Amplex™ Red Sucrose Assay file (Amplex Sucrose.qbt) into the USB port on the instrument. The device does not need to be restarted.

2.3 From the Home Screen, select **Settings**.

2.4 In the "Settings" window, select **Import New Assay** and follow the on-screen prompts.

Critical assay parameters

Incubation time

The MyQubit Amplex™ Red Sucrose assay for use with the Qubit™ Fluorometer was optimized using Amplex™ Red/UltraRed Stop Reagent. This reagent provides a means to terminate the fluorescence signal-generating reaction in the enzymatic assay. Once the stop reagent has been added, 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.

Qubit™ Fluorometer calibration

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.

Calculation of sample concentration

The Qubit™ Fluorometer gives values for the MyQubit Amplex™ Red Sucrose 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:

$$\text{Concentration of your sample} = \text{QF value} \times \frac{241}{x}$$

where QF value is the value given by the Qubit™ 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™ Fluorometer for the dilution in the assay tube. Because the MyQubit Amplex™ Red Sucrose assay gives concentrations in µM, the result of the equation above will be in µM.

IMPORTANT! The original sample calculator function (called Dilution Calculator on the Qubit™ 2.0 Fluorometer) found in other Qubit assays is not applicable to MyQubit assays. This function was designed specifically to accommodate sample dilutions of 1–20 µL in a final assay volume of 200 µL. These conditions do not apply to this assay.

Experimental procedure

Prepare stock solutions

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. Where possible, prepare aliquots of the stock solutions for sucrose, glucose oxidase, invertase, HRP, and the Amplex Red™ reagent to avoid unnecessary freeze-thaw cycles.

3.1 Prepare a 10 mM stock solution of Amplex™ Red reagent. Allow one vial of Amplex™ Red reagent (Component A, 154 µg, blue cap) and DMSO (Component B, green cap) to warm to room temperature. Just prior to use, dissolve the contents of the vial of Amplex™ Red reagent in 60 µL of DMSO. Each vial of Amplex™ Red reagent is sufficient for approximately 50 assays.

3.2 Prepare 1X Reaction Buffer. Add 4 mL of 5X Reaction Buffer (Component C, 0.25 M sodium phosphate pH 7.4, white cap) to 16 mL of deionized water (dH_2O). This 20 mL volume of 1X Reaction Buffer is sufficient for approximately 50 assays of 200 µL each with a 10 mL excess for preparing stock solutions. Refrigerate the 1X Buffer Solution at 2–8°C. Do NOT freeze the 1X Buffer Solution.

3.3 Prepare a 10 U/mL stock solution of horseradish peroxidase (HRP). Dissolve the contents of the vial of HRP (Component D, 10 U, yellow cap) in 1 mL of 1X Reaction Buffer. After the assay, divide any remaining unused solution into single-use aliquots and store frozen at ≤–20°C.

3.4 Prepare a 100 U/mL stock solution of glucose oxidase. Dissolve the contents of the vial of glucose oxidase (Component E, 100 U, orange cap) in 1.0 mL of 1X Reaction Buffer. Store this stock solution frozen at ≤–20°C.

3.5 Prepare a 5000 U/mL invertase stock solution. Weigh out 12.5 mg of invertase (100 U/mg), and dissolve it in 250 µL of 1X Reaction Buffer. Store this stock solution frozen at ≤–20°C.

3.6 Prepare an 80 mM (27.3 mg/mL) sucrose stock solution. Weigh out a portion of sucrose, and dissolve it in the appropriate amount of 1X Reaction Buffer. Store this stock solution frozen at ≤–20°C.

3.7 Prepare 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. Aliquot the solution as needed and dilute with dH_2O in a 1:1 ratio. This amount is sufficient to stop 70 assays using 40 µL for each 200 µL assay tube. 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.

Perform the MyQubit Amplex™ Red Sucrose assay

The protocol below describes the MyQubit Amplex™ Red Sucrose assay in a total volume of 240 µL per tube. This volume includes the Amplex™ Red/UltraRed Stop Reagent used to quench the enzymatic reactions (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 Amplex™ Red Glucose/Glucose Oxidase Assay Kit contains sufficient reagents for 250 sucrose determinations using the volumes recommended here.

Table 1 Volume of reagents used in the MyQubit Amplex™ Red Sucrose assay

	Standard Assay Tube	User Sample Assay Tube
Volume of **Standard** (or blank)	100 µL	—
Volume of **User Sample**	—	100 µL
Volume of **Invertase**	1 µL	1 µL
Volume of **Working Solution**	100 µL	100 µL
Volume of **Stop Reagent**	40 µL	40 µL
Total volume in each Assay Tube	241 µL	241 µL

If you suspect that the sample contains glucose, we recommend running the MyQubit Amplex™ Red Glucose Assay prior to the Sucrose Assay. If glucose is indeed present, prepare an additional sample vial. Follow all the instructions for the sucrose analysis, but do NOT add invertase, and label the tubes accordingly (e.g., 1a, 1b, 1c, etc.). This will allow you to determine the signal associated with free glucose in the sample. This is not meant to be the same value as the one obtained from the MyQubit Amplex™ Red Glucose assay as the two programs utilize slightly different curve fit parameters.

After the analysis is complete, subtract the reported concentration of the invertase free sample (e.g., 1a) from the experimental sample to determine the sucrose concentration in the experimental sample (e.g., 1):

Reported Sample Concentration (sample 1) – Invertase Free Sample Concentration (sample 1a) = Sucrose Concentration in Sample.

This approach works best when the sucrose concentration is greater than half of the glucose concentration. If further treatment is needed, wherein the sucrose concentration of the analyte is less than twice the glucose concentration, use the protocols outlined in the Appendix.

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 Sucrose Assay and the Glucose Assay kit, the procedure described below should be used for performing the assay.

Note: MyQubit Amplex™ Red Sucrose assay calibration requires a final concentration of 100 µM sucrose in the Standard 2 assay tube (where the final volume is 240 µL).

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

4.1 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 (Thermo Fisher Scientific, Cat. no. 14-222-292).

4.2 Dilute sucrose containing samples into 1X Reaction Buffer. For new users, we recommend preparing a series of sample dilutions in order to ensure that the sample will fall within the detection range of the assay (a final concentration of 0.25 to 100 µM sucrose). Prepare dilutions with the 1X Reaction Buffer and label them accordingly (e.g., 1, 2, 3, etc.). Add 100 µL of each solution to an assay tube and label it accordingly.

4.3 Prepare calibration standard 1. Add 100 µL of 1X Reaction Buffer to one assay tube and label it accordingly.

4.4 Prepare calibration standard 2. For calibration, a final concentration of 100 µM sucrose is needed. Into a new microfuge tube, add 3 µL of the 80 mM sucrose stock solution and dilute it with 997 µL of 1X Reaction Buffer. This 1 mL of 240 µM sucrose solution is sufficient to perform 10 standard calibrations. Add 100 µL of this solution to a second assay tube and label it accordingly. Store any unused portion of the standard at ≤–20°C.

4.5 Add invertase solution and mix thoroughly. To each sample tube, add 1 µL of the invertase solution. Vortex all of the tubes for 2–3 seconds, and then continue mixing/agitating for 15 minutes.

4.6 Prepare Working Solution. Add the following reaction components in the order presented and mix thoroughly:

950 µL of 1X Reaction Buffer

20 µL of HRP stock solution (working solution concentration: 0.2 U/mL)

20 µL of glucose oxidase stock solution (working solution concentration: 2 U/mL)

10 µL of Amplex™ Red reagent stock solution (working solution concentration: 100 µM Amplex™ Red reagent)

Note: This 1 mL volume is sufficient for ~10 assays. The final concentration of each component will be two-fold lower in the final reaction volume. For best results, use the working solution promptly once the Amplex™ Red reagent has been added. If the solution turns pink upon addition of the Amplex™ Red reagent, the working solution has been contaminated and should be remade.

4.7 Start the reactions. Add 100 µL of the Working Solution to each assay tube containing standard or sample. Try to add the working solution to all assay tubes at the same time; if possible, use a multi-channel or repeat pipettor. For best results, use a new pipette tip for each sample and thoroughly mix upon addition.

4.8 Incubate. Incubate the samples at room temperature for 15 minutes, protected from light. For best results, mix/agitate the samples continuously during incubation.

4.9 Terminate the reaction. After incubation is completed, add 40 µL of the prepared Amplex™ Red/UltraRed Stop Reagent into each assay tube (standards and samples) and vortex all of the tubes for 2–3 seconds.

4.10 Run the MyQubit Amplex™ Red Sucrose assay. On the Qubit™ Fluorometer, select **Sucrose** from the Home Screen and follow the on-screen instructions. As prompted, first read the calibration standard 1 solution, followed by the calibration standard 2 solution. This will set the standard curve for analysis of the unknown samples. After the standards are read, read the unknown samples.

Note: When reading, make sure that there is not a significant volume of standard/sample left in the cap of the assay tube.

IMPORTANT! When reading samples on the Qubit™ 3.0 Fluorometer, you will be prompted to "Enter the original sample volume." Because the dilution calculator does not apply to MyQubit assays, enter any value between 1–20 µL to proceed. On the following screen, read your sample concentration from the lower circle, "Qubit Tube Concentration." The value in the upper circle is determined using the dilution calculator and is not valid for your sample.

4.11 Calculate the sample concentration. Reported concentrations are in µM and reflect the concentration of the sample inside the assay tube at a volume of 241 µL. To calculate the concentration of your starting sample, use the following equation:

$$\text{Concentration of your sample} = \text{QF value} \times \frac{241}{x}$$

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. The presence of glucose in the sample will lead to a false/inflated analysis. Therefore, always run the MyQubit Amplex™ Red Glucose assay prior to running the MyQubit Amplex™ Red Sucrose assay.

   - If the glucose concentration in the sample is above 12.5 µM, or the ratio of glucose to sucrose is greater than 2:1, the obtained data will not be reliable. In this situation, we recommend removing the glucose using the protocol in Appendix 2 (page 9).
   - If the glucose concentration in the sample is not over this limit and not negligible, removing the glucose is not necessary, but an additional control will need to be run. In this situation, follow the modified protocol below:

     Prepare an additional vial per analysis sample. Follow all the instructions for the sucrose analysis, but do NOT add invertase and label it accordingly. This will allow you to determine the amount of free glucose in the sample. After the analysis is complete, subtract the reported concentration of the invertase-free sample from the experimental sample to determine the sucrose concentration in the experimental sample:

     Reported Sample Concentration – Invertase-Free Sample Concentration = Sucrose Concentration in Sample.

2. To remove glucose from the sample, follow the instructions below. To do this, you will need catalase (Fischer, Cat. no. S25239) and hydroxylamine (Acros Organics, Cat. no. AC412051000).

   a. Prepare a 40 mM solution of hydroxylamine hydrochloride using the 5X buffer as the solvent.

   b. Prepare a 20,000 Unit/mL solution of catalase using the 1X buffer.

   c. In a 2 mL microfuge tube, combine 10 µL of the catalase solution, 10 µL of the glucose oxidase solution, and 30 µL of 5X buffer (total volume of 50 µL in tube).

   d. To the microfuge tube, add 100 µL of the sample and mix thoroughly (total volume of 150 µL in tube). Approximately 20% of the original glucose signal will remain after 1 minute and less than 1% will remain after 60 minutes.

   e. Quench the reaction with 50 µL of the 40 mM hydroxylamine solution (total volume of 200 µL in tube). Mix thoroughly. The quenching reaction is instantaneous.

   f. Remove 100 µL from the microfuge tube containing 200 µL of quenched reaction mixture. Use this aliquot for the sucrose determination, noting that it has been diluted 2X.

   Note: To be more thorough, you can run a glucose signal control alongside the sucrose determination. In this case, add an additional 50 µL of 1X buffer to the microfuge tube before removing your aliquot (step f). Now, with a total volume of 250 µL, remove 100 µL for the two experimental tubes, noting a relative dilution of 2.5X.

3. 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 sucrose. 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 Sucrose 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™ 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, if you omit the stop reagent, we recommend customizing the parameters in the Amplex Sucrose.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 the "Raw Mode" (on Qubit™ 2.0 instruments) or the "Fluorometer Mode" (on Qubit™ 3.0 instruments) to collect raw fluorescence data over multiple time points to measure the kinetics of the reaction.

4. The Amplex™ Red Glucose/Glucose Oxidase Assay Kit has also been shown to be compatible with the Amplex™ UltraRed reagent. The Amplex™ UltraRed reagent (Cat. no. A36006) provides all of the same performance characteristics 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 the Amplex™ UltraRed reagent. However, for best results, we recommend optimizing the parameters in the Amplex Sucrose.qbt file for use with the Amplex™ UltraRed reagent. For detailed instructions on how to create a custom assay using MyQubit, refer to **www.lifetechnologies.com/qubit**.

5. If your analysis method permits, you may attain increased sensitivity using the Amplex™ UltraRed reagent and a 1X Buffer Solution at pH 6.5. However, the standard curve on the MyQubit Amplex™ Red Sucrose assay was not optimized for this. If this is of interest, we recommend optimizing the parameters in the Amplex Sucrose.qbt file using MyQubit as noted in Appendix 3.

6. You can desiccate and store prepared working solution without compromising the sensitivity of the assay. However, this can only be done before the Amplex™ Red reagent is added as it requires DMSO for solubility. For this, prepare the working solution as noted above in step 4.6 (page 7), but do NOT add the Amplex™ Red reagent. Concentrate the solution by spin vacuum or freeze drying. To reconstitute, add 990 µL of E-pure H_2O and 10 µL of the Amplex™ Red reagent stock solution, and mix thoroughly.

7. For best results, label the Qubit™ Assay Tubes on the top of the tube as labels on the side of the tube may interfere with the fluorescence readings.

8. Depending on the workflow, it may be desirable to aliquot the Amplex™ Red reagent into single-use vials to prevent repeated freeze-thaw cycles. To do this, we recommend diluting the sample into acetonitrile, aliquotting the necessary volume into new tubes, and concentrating until dry using a spin-vacuum.

References

1. Proc SPIE-Intl Soc Opt Eng 3606, 119 (1999); 2. Anal Biochem 253, 162 (1997).

Product list

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

Cat. no.	Product name	Unit size
Q33216	Qubit™ 3.0 Fluorometer	each
A22189	Amplex™ Red Glucose/Glucose Oxidase Assay Kit	1 kit
A33855	Amplex™ Red/UltraRed Stop Reagent	1 set
A36006	Amplex™ UltraRed Reagent	5 × 1 mg
Q32856	Qubit™ Assay Tubes	500 tubes

Purchaser notification

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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