

# Take3™ Multi-Volume Plate


## User Guide





# **Take3™ Multi-Volume Plate**

## **User Guide**



September 2009

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Manual Part Number 4691000

Revision B

BioTek® Instruments, Inc.

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### Technical Assistance Center ("TAC")

TAC is open from 8:30 AM to 5:30 PM (EST), Monday through Friday, excluding standard U.S. holidays.

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## Intended Use Statement

The Take3 Multi-Volume Plate can be used in compatible BioTek detection systems to perform measurements with very low (2  $\mu$ L) sample volumes. The Take3 plate can also be used to measure samples in the BioTek BioCell and/or stoppered cuvettes.

This product may be used for In Vitro Diagnostic, research and development, or other non-clinical purposes

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## CE Mark



Based on the programs described below and information contained herein, this product bears the CE mark.

❖ See the Declaration of Conformity for more information.

### Directive 98/79/EC: In Vitro Diagnostics

Risk Assessment conducted to: ISO 14971 – Medical devices – Application of risk management to medical devices

BioTek registration to: ISO 13485 – Medical devices – Quality management systems – Requirements for regulatory purposes

Microplate Reader registration with competent authorities.

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## Warranty and Product Registration

Review the Warranty information that shipped with your product.

Register your product(s) with BioTek to ensure that you receive important information and updates. Contact the Customer Resource Center (CRC) at [www.biotek.com](http://www.biotek.com) or by calling 888/451-5171 or 802/655-4740.

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## Warnings, Hazards, and Precautions



Measurements are affected by extraneous particles (such as dust or lint) on the glass slides. A clean work area is essential to ensure accurate readings.

When operated in a safe environment according to the instructions in this document, there are no known hazards associated with the Take3 plate. The user should be aware of certain situations that could, however, result in injury or damage to the plate. Read the following hazards and precautions.

The following **hazard** warnings help avoid injury:



**Warning! Potential Biohazards.** Some assays or specimens may pose a biohazard. Adequate safety precautions should be taken as outlined in the assay's package insert. Always wear safety glasses and appropriate protective equipment, such as chemical-resistant rubber gloves and apron.



**Warning! Sharp edges.** The corners of the glass slides can be sharp. Use a pipette tip to push the slide away from the plate for removal. Always wear gloves, and handle carefully.

**Warning! Pinch Hazard.** When replacing slides avoid pinching a finger between the glass and the plate by setting one short end of the slide into its housing and then laying the slide flat.

**Warning!** Mucous membranes are considered prime entry routes for infectious agents. Wear eye protection and a surgical mask when there is a possibility of aerosol contamination. Intact skin is generally considered an effective barrier against infectious organisms; however, small abrasions and cuts may not always be visible. Wear protective gloves when handling a contaminated Take3 plate.

**Warning!** Gloved hands should be considered contaminated at all times; keep gloved hands away from eyes, mouth, nose, and ears. Eating and drinking while decontaminating the Take3 plate is not advised.

**Warning!** The bleach solution is caustic; wear gloves and eye protection when handling this solution.



**Warning!** The plate contains magnets and should be kept away from other magnetic media and sensitive electronic devices.

The following **precautions** help avoid damage to the Take3 plate:



Proper care and handling of this precision measurement device is essential to retain its value. Store the plate in its case or other secure location when not in use. Keep the plate clean and free of dust. Do not drop the plate!

Do not expose any part of the plate to the recommended diluted sodium hypochlorite solution (bleach) for more than 20 minutes. Prolonged contact may damage the plate. Rinse and thoroughly wipe all surfaces.

Do not autoclave the Take3 plate or glass slides.

To avoid scratching the Teflon coating on the microspot slide, do not use anything sharp or abrasive when cleaning.

Do not soak the plate. In particular, bleach and other harsh cleansers can corrode the plate hinge.

Do not use bleach or corrosive cleansers on the sides of the slides with the metal disks.

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## Document Revision History

Revision	Date	Changes
A	09/2009	First Release
B	09/2009	Update Optional Accessories list. Add test solution available from BioTek for calibrating the plate pathlengths.



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# Take3 Multi-Volume Plate

## Overview

BioTek's Take3 Multi-Volume Plate allows quick and efficient nucleic acid and protein quantification by three different methods: very low volume (2  $\mu$ L) sample measurements, BioCell 1 cm measurements, and standard cuvette measurement.

Measurements are made using compatible BioTek absorbance microplate readers, using the Gen5 Take3 software module. The module contains pre-programmed applications, including:

- **Nucleic Acid Quantification:** dsDNA Concentration, RNA Concentration, ssDNA Concentration
- **Protein A280:** BSA, IgG, Lysozyme

The Gen5 Take3 module performs the calculations and exports the results to a Microsoft Excel spreadsheet. Results include raw ODs, ratio and calculated concentrations for each sample, and blank measurement results.

For further flexibility, Take3 can be used with a standard protocol in the Gen5 microplate interface for assays including spectral scans and qualitative and quantitative analysis with 2  $\mu$ L samples.

## Plate Description

The Take3 plate is dimensionally similar to standard 96-well microplates and contains:

- 16 2-mm diameter 2  $\mu$ L "microspots" arranged in a 2x8 geometry (locations A2-H3),
- two locations for BioCells (A9, H9); and
- two reading locations (E10, E11) for one stoppered rectangular cuvette with a maximum length of 54 mm overall.

Access the microspots by opening the plate lid and exposing a Teflon-coated fused-silica glass slide (Figure 2).

After pipetting samples, gently close the lid (which also has a glass slide) to form a consistent 0.5 mm pathlength (Figure 3).



Figure 1: Plate closed, showing BioCells and cuvette



Figure 2: Plate open, showing slides and "microspots"

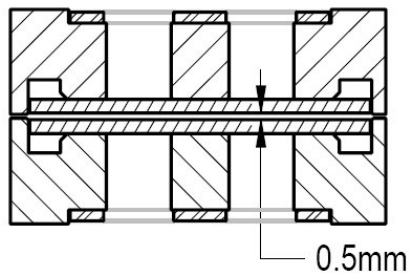


Figure 3: Cross-section of microspots showing top and bottom glass slides with nominal 0.5 mm pathlength

## Package Contents

Storage case containing:

- Take3 Multi-Volume Plate and User Guide
- Gen5 Take3 Module and Quick Start Guide
- Data sheet with plate-specific pathlength calibration data
- Two pockets for storing BioTek's BioCell quartz vessels
- Two pockets for storing cuvettes

If the case or plate arrives damaged, notify the carrier and your BioTek representative. Keep the shipping box and any packing material for the carrier's inspection. BioTek will repair or replace your product immediately.

Before using the plate, remove the protective **foam sheet** from between the glass slides. Store the foam in the storage case for use when shipping the plate back to BioTek or to protect the slides when the plate is not in use.

## Optional Accessories from BioTek

- BioCell quartz vessel (PN 7272051)
- 10 mm quartz cuvette with stopper (PN 48723)
- Replacement slide kit (PN 4690009)

## Compatible BioTek Readers

The Take3 plate is supported by several BioTek monochromator-based absorbance readers, including Epoch, PowerWave XS/XS2, and all Synergy readers equipped with absorbance detection. Contact BioTek for more information.

## Software Requirements

Gen5 or Gen5 Secure version 1.09 or greater must be installed on your computer to run the Gen5 Take3 Module. Microsoft Excel is also required, for results reporting. Refer to the **Gen5 Take3 Module Quick Start Guide** for details.

## Returning the Plate to BioTek

If you need to return the plate for repair or replacement:

- 1 Contact BioTek TAC for a Return Materials Authorization (RMA) number (p. 4).
- 2 Decontaminate the plate (p. 19).
- 3 Insert the protective foam sheet between the glass slides.
- 4 Place the plate and any accessories in the original storage case.
- 5 Package the storage case in an appropriately sized padded shipping box.
- 6 Send to:

BioTek Instruments, Inc.  
ATTN: RMA# xxxxx  
100 Tigan Street  
Highland Park  
Winooski, Vermont 05404 USA



**Warning!** U.S. Department of Transportation regulations require decontamination prior to shipping. If the product has been exposed to potentially hazardous material, decontaminate it to minimize the risk to all who come in contact with the plate during shipping, handling, and servicing.

# Getting Started

## 1: Install the Software

Measurement collection, data reduction, and results output are performed using BioTek's Gen5 software with the Gen5 Take3 Module. Refer to the installation instructions in Gen5's **Getting Started Guide** and **Take3 Module Quick Start Guide** as necessary.

## 2: Set Up the Reader

Refer to the reader's Operator's Manual to install the reader and verify communication with Gen5.

## 3: Configure the Gen5 Take3 Module

Before the plate is used for the **first time**, use the Take3 module to:

- ❖ Run a **calibration** procedure on the intended reader to capture the positions of the Take3 plate's microspots.
- ❖ Enter **pathlength** values associated with your plate's microspots for use with calculating concentrations.

- 1 Turn on the reader and start Gen5.
- 2 In Gen5, select **Take3 > Take3 Plates**, and click **New**.
- 3 Enter the Take3 plate's **Serial Number**.
- 4 Place the plate on the microplate carrier. Align 'A1' on the plate with 'A1' on the carrier.
- 4 Click **Calibrate, Start Calibration**, and **OK**. The process takes several minutes. When finished, click **OK**.
- 5 Locate the **Take3 Pathlengths Data Sheet** that came with the plate. Gen5's Pathlengths table contains 16 values, for locations A2-H3. Replace the default 0.5 mm values with those from the data sheet. When finished, click **OK**.

**Take3 Plate**

Serial Number:

**Dimensions**

The plate must be calibrated before use.

**Pathlengths (mm)**

	2	3
A	0.5	0.5
B	0.5	0.5
C	0.5	0.5
D	0.5	0.5
E	0.5	0.5
F	0.5	0.5
G	0.5	0.5
H	0.5	0.5

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## Preparing the Plate



Proper care and handling of this precision measurement device is essential to retain its value. Store the plate in its case or other secure location when not in use. Keep the plate clean and free of dust.

Do not touch the glass slides with bare fingers.

Samples containing detergent or other surfactant may not be suitable for low-volume microspot measurements.

After pipetting samples into the microspots, lower the plate lid *gently* to avoid splashing.

Pipette samples efficiently and read the plate immediately to avoid evaporation.

### Prepare a Gen5 Take3 Session

We recommend preparing a Gen5 Take3 session *before* pipetting samples.

- To get started, launch Gen5 and select **New Take3 Session** from the Welcome screen or the File menu.
- Double-click on the desired application type on the left.
- Select the Take3 Plate, Well Type, and Sample Type.
- Click the Help button for assistance.

### Microspots

You will need:

- A calibrated single- or multi-channel pipette capable of accurately pipetting 2  $\mu\text{L}$ , and 0.1-10  $\mu\text{L}$  pipette tips
  - Lint-free disposable paper wipes to clean the slides between measurements
- 1 Open the plate lid to access the microspots.
  - 2 Pipette up to 16 samples on the slide (see photos on page 14).
  - 3 When finished, lower the plate lid gently to avoid splashing the samples.
  - 4 Read the plate immediately (p. 15).

- 5 After reading the plate, clean and test the slides before pipetting fresh samples (p. 16).

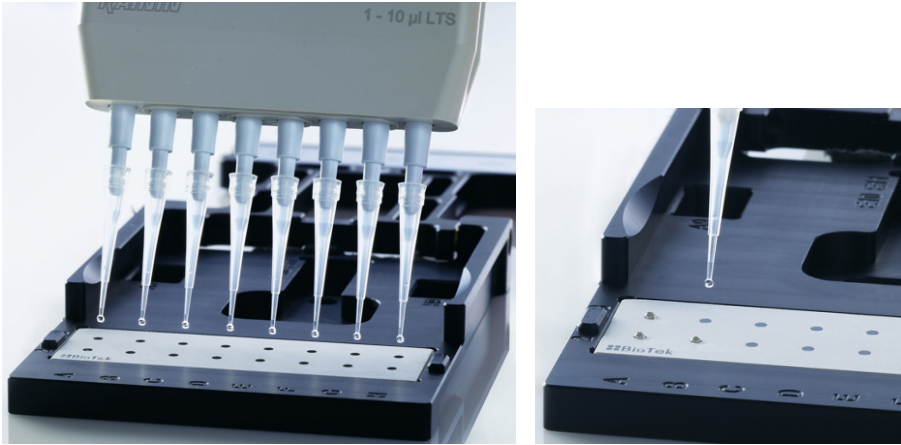


Figure 4: Pipetting samples

## BioCell

- The Take3 plate supports up to two BioCells (available from BioTek) in plate locations A9 and H9 (Figure 1, p. 9).
- Ensure that the BioCell is installed with the port to the right in the plate. If the port is capped, remove the cap prior to measurement.

## Cuvette

- The Take3 plate supports one cuvette, which must be rectangular, stoppered, and no longer than 54 mm. Low volume cuvettes can be used if they have the same physical dimensions as full volume cuvettes.
- Place the cuvette into the plate with the stopper to the left and the transparent side of the cuvette face up (Figure 1, p. 9).
- Ensure that plate locations E10 and E11 do not contain air bubbles.
- When measuring a low volume cuvette, use plate location E10.

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## Reading the Plate

Brief procedures for using Gen5 are provided below. Refer to the Gen5 Help system for detailed instructions.

### Using the Gen5 Take3 Module

- 1 If you have not already done so, prepare a Take3 Session (p. 13).
- 2 Prepare the plate (p. 13).
- 3 Place the plate on the reader's microplate carrier. Align 'A1' on the plate with position **A1** on the carrier.
- 4 In the Gen5 Take3 module:
  - Select the blank wells to be read. Click **Read Blanks** and then click **OK**.
  - Select the sample wells to be read. Click **Read Samples** and then click **OK**.
  - When finished, Microsoft Excel will launch and display the results (you may need to click **End of Batch**).

### Using the Gen5 Microplate Interface

- 1 From Gen5's main view select **File > New Protocol** and then **Protocol > Procedure**.
- 2 Set the Plate Type to Take3 (<your plate's serial number>).
- 3 Click the **Read** button and define the reading parameters. Click the **Full Plate** button in the upper-right corner to select the wells to be read.
- 4 Return to the main view and select **File > Save** to save the protocol.
- 5 Select **File > New Experiment**. Highlight the protocol name and click **OK**.
- 6 Prepare the plate (p. 13).
- 7 Place the plate on the reader's microplate carrier.
- 8 Select **Plate > Read** and click **READ**. Respond to any additional prompts that appear.
- 9 When finished, review the data and select **File > Save** to save the experiment.



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## Cleaning

The Take3 plate is a precision measurement device. Keep the plate and slides clean and free of debris to ensure accurate results.

**Caution:**

- Do not autoclave the plate or glass slides.
- Do not use corrosive cleansers. Do not soak the plate.
- To avoid scratching the Teflon coating on the glass, do not use anything sharp or abrasive when cleaning.
- After cleaning, do not touch the glass with bare fingers.

Between measurements, the slides can usually be cleaned in place using dry **lint-free** disposable paper wipes. When more thorough cleaning is required, the slides can be removed and cleaned using a mild detergent.

### Quick Clean

With the slides in place:

- Use one dry laboratory wipe to **blot** the fluid from both slides and use another to **wipe** off any remaining fluid.
- If necessary, use clean canned air to remove fine debris.
- (Optional, but recommended) Before pipetting fresh samples, verify the cleanliness of the slides (p. 21).

### Thorough Clean

The slides can be removed from the plate and cleaned using a mild detergent. After replacing the slides, we recommend calibrating the plate pathlengths to ensure the most accurate measurements (p. 22)

For this procedure you will need a mild detergent or Tween® 20 (polyoxyethylene (20) sorbitan monolaurate), deionized water, a pipette tip, 70% isopropyl alcohol or ethanol (optional), and an incubator (optional).

- ❖ Wear lint-free gloves and handle the slides carefully. The glass edges may be sharp.

- 1 Prepare a mixture of soapy water or 2% Tween and deionized water.

- The slides are held in place with magnets. To remove a slide, use a pipette tip to push the slide away from the plate.



Figure 5: Using a pipette tip to remove a slide

- (Optional) Moisten a lint-free laboratory wipe with alcohol and wipe each slide on its flat surface only. Do not wipe the metal disks with alcohol.

❖ A white residue may appear if alcohol touches the glue around the metal disks. The residue can be removed with soapy water, using a soft brush if necessary.

- Moisten a laboratory wipe with the cleaning solution and wipe the slides on both sides.
- Rinse the slides in deionized water and dry them with a laboratory wipe.
- Air-dry the slides or dry them in an incubator (40° to 60° C). If necessary, use clean canned air to remove debris.
- If necessary, wipe the **plate** with the cleaning solution before replacing the slides:
  - Wipe the three contact points (Figure 6) and their counterparts on the lid, the stainless-steel plates, and the rest of the plate.

❖ If debris accumulates on the contact points the lid may not close properly, which can affect measurement accuracy.

- Wipe the entire plate with a lint-free cloth moistened with deionized water, and then dry all wet surfaces using laboratory wipes.

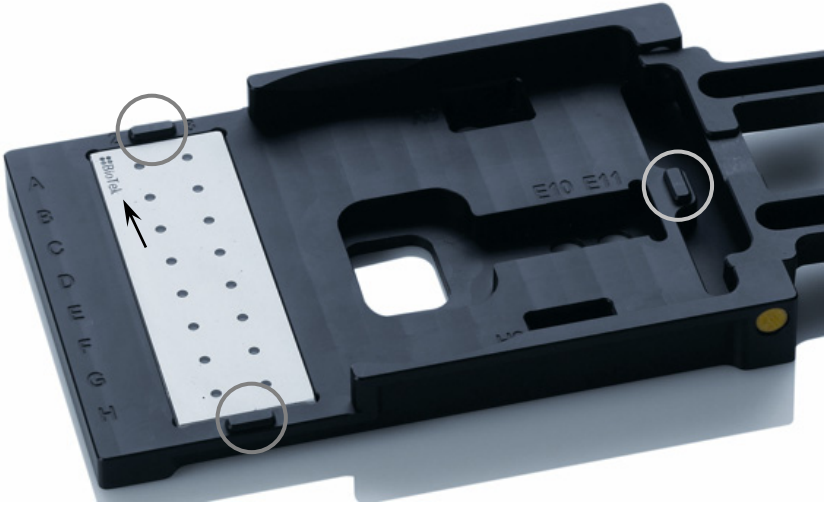


Figure 6: Three contact points and BioTek logo

8 Replace the slides:



- To avoid pinching your fingers, set one short end of the slide into its housing and then lay the slide down.
- Ensure that all three disks contact the magnets and the slide lies flat.
- Orient the microspot slide with the BioTek logo near location A2 (as shown above).

9 Before pipetting fresh samples:

- Verify the cleanliness of the slides (p. 21).
- Calibrate the plate pathlengths to ensure the most accurate measurements (p. 22).

## Decontamination

Perform this procedure before storing the plate or sending it to BioTek.

	<p><b>Warning!</b></p> <p>Mucous membranes are considered prime entry routes for infectious agents. Wear eye protection and a surgical mask when there is a possibility of aerosol contamination. Intact skin is generally considered an effective barrier against infectious organisms; however, small abrasions and cuts may not always be visible. Wear protective gloves when handling contaminated instruments.</p> <p>The bleach solution is caustic; wear gloves and eye protection when handling this solution.</p>
	<p><b>Caution:</b></p> <p>Check the percent NaClO of the bleach you are using; this information is printed on the side of the bottle. Commercial bleach is typically 10% NaClO; prepare a 1:20 dilution. Household bleach is typically 5% NaClO; prepare a 1:10 dilution.</p> <p>Do not prepare a stronger bleach solution than described here. Extended exposure to high concentrations of bleach can deteriorate the plate surfaces.</p> <p>Do not soak the plate or slides in bleach or other harsh cleansers.</p> <p>Do not autoclave the plate or slides.</p>

- 1 Prepare an aqueous solution of 0.5% sodium hypochlorite (NaClO, or bleach). If the effects of bleach are a concern, use a mild detergent or 2% Tween 20 in deionized water.
- 2 The slides are held in place with magnets. Remove both slides, using a pipette tip to push the slide away from the plate (p. 17).
- 3 Moisten a clean, lint-free cloth with the bleach solution or soapy water.

If using bleach:

- Wipe the slides on the sides without the metal disks.
- Wipe the entire plate.

- Wait 20 minutes.
- Moisten a cloth with deionized or distilled water. Wipe the slides and plate thoroughly to remove all bleach.
- Wipe the slides and plate with a clean, dry cloth to dry all wet surfaces.
- Air-dry the slides or dry them in an incubator (40 to 60° C).

If using soapy water:

- Wipe the slides on both sides.
- Wipe the entire plate.
- Moisten a cloth with deionized or distilled water. Wipe the slides and plate thoroughly to remove all solution.
- Wipe the slides and plate with a clean, dry cloth to dry all wet surfaces.
- Air-dry the slides or dry them in an incubator (40 to 60° C).

4 Replace the slides:

- To avoid pinching your fingers, set one short end of the slide into its housing and then lay the slide down.
- Ensure that all three disks contact the magnets and the slide lies flat.
- Orient the microspot slide with the BioTek logo near location A2 (p. 18).

5 Before pipetting fresh samples:

- Verify the cleanliness of the slides (p. 21).
- Calibrate the plate pathlengths to ensure the most accurate measurements (p. 22).

6 Discard the cleaning materials in an approved biohazard container.

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# Verification and Calibration

This section describes procedures for (a) verifying the cleanliness of the slides, and (b) calibrating pathlengths for the microspots when the slides are replaced after cleaning.

## Gen5 Protocol Setup

The Gen5 Take3 module installs the protocols referenced in the following procedures. Before a protocol can be used for the first time, it must be modified and saved for use with your Take3 plate.

- 1 Open the file in Gen5 (select **File > Open Protocol** and navigate to \Gen5 Utilities\Take3 Protocols).
- 2 Select **Protocol > Procedure**. A 'Take3 (SN)' warning message will appear; click **OK**.
- 3 At the top of the Procedure dialog, click the **Plate Type** box and select 'Take3 (<your plate's serial number>)'.
- 4 Click **OK** and then select **File > Save As**.
- 5 (Optional) Insert the serial number into the file name (e.g., Blank Plate SN116449.prt), and click **Save**.

## Verifying the Cleanliness of the Slides

After cleaning the slides (p. 16), we recommend that you read the microspots at 260/320 nm to check for any residue that may affect your measurements.

- 1 If you have not already done so, modify the **Blank Plate.prt** protocol as described above.
- 2 Place the Take3 plate in the reader's microplate carrier.
- 3 Start Gen5 and create an Experiment based on the **Blank Plate** protocol (**File > New Experiment**).
- 4 Select **Plate > Read**, click **READ**, and then click **OK**.
- 5 When the read is complete, select **Plate > Print** to print the report (or **Plate > PowerExport** to view it in Excel).
- 6 We recommend cleaning and verifying the slides again if any Delta OD values are greater than approximately 0.020. (Clean slides *typically* measure less than 0.012 OD.)

## Calibrating the Plate Pathlengths

When replacing the slides after cleaning or decontamination, perform a calibration procedure to compensate for any deviation from the nominal 0.5 mm pathlength between the slides (see **Method** below details). The procedure can be performed using a single- or multi-channel pipette with a cuvette or BioCell. Gen5 provides four protocols to cover any combination of pipette and cuvette or BioCell.

Before starting: If you have not already done so, modify **Check Solution.prt** and the appropriate **Pathlength Calibration** protocol using the **Gen5 Protocol Setup** procedure on page 21.

### Method

The Take3 plate is designed with nominal pathlengths of 0.5 mm for each of 16 microspots. In reality each microspot has a slightly different pathlength and can vary from the intended 0.5 mm. During the calibration procedure, the exact pathlength is determined for each microspot and stored in the Gen5 Take3 module for determining the concentration of solutions based on optical density measurements.

The calibration method compares the optical density of two solutions with a known concentration ratio. Solution **C1** has a high concentration of yellow dye. Solution **C2** is diluted from C1 ( $C2=C1/20$ ).

When performing the procedure, you will pipette 5  $\mu$ L of C1 into each microspot, fill a 10 mm cuvette or 1 cm BioCell with C2, and then run a Gen5 experiment to measure the microspots and cuvette or BioCell at 428-530 nm. Gen5 performs the pathlength calculations automatically; a brief explanation follows.

Optical density is proportional to the product of concentration and optical pathlength, thus:

$$\begin{aligned} \text{OD\_microspot} &= K \cdot C1 \cdot (\text{pathlength of microspot}) \\ \text{OD2\_cuvette} &= K \cdot C2 \cdot (\text{pathlength of cuvette or BioCell}) \end{aligned}$$

From these two equations it follows that:

$$(\text{pathlength of microspot}) = \text{OD\_microspot} \cdot C2 \cdot (\text{pathlength of cuvette}) / (\text{OD\_cuvette} \cdot C1)$$

or, written another way:  $(\text{pathlength of microspot}) = (\text{OD\_microspot} / \text{OD\_cuvette}) \cdot (C2 / C1) \cdot 10 \text{ mm}$

## Materials

- BioTek Calibration Solution (PN 4693002) **OR** FD&C Yellow No. 5 Powder and HPLC grade water (if available, otherwise deionized or distilled water)
- 0.5 - 10  $\mu$ L single- or multi-channel pipette with 0.1 - 10  $\mu$ L pipette tips
- Drummond pipette aid (or equivalent) with 25 mL disposable serological pipette
- 1 mL pipette
- 100 mL storage bottle with cap
- Two 50 mL conical tubes with caps
- Disposable pipette troughs
- Lint-free disposable paper wipes
- BioCell (PN 727051) or 10 mm cuvette with stopper (BioTek offers a quartz cuvette, PN 48723)
- Beaker or other small container
- Scale readable to 0.0001 g
- Weigh paper
- 0.2  $\mu$ m filter

## Solutions

### Mix the Start Solution (if not using BioTek's Calibration Solution):

- 1 Add 0.020 g of yellow dye power to 50 mL of water. Mix until dissolved.
- 2 Filter the solution into a 50 mL tube. Cap the tube when not in use to minimize evaporation.
- 3 In Gen5, create an experiment based on the **Check Solution** protocol.
- 4 Pipette 5  $\mu$ L of the Start Solution onto microspot A2. Close the plate lid immediately.
- 5 Place the plate on the carrier and run the experiment. When finished, select **Plate > Print** to print the report (or **Plate > PowerExport** to view it in Excel).
- 6 The **Delta OD** value should be in the range 0.8 to 1.2 OD.



**Mix the 20:1 Solution:**

- 1 Place a 50 mL tube (without the cap) in a glass beaker on the balance. Tare the balance.
- 2 Use a 25 mL pipette to dispense 19 mL of water into the tared tube. The weight should be in the range 18.500 – 19.500 g. Note this value as the '**Weight of Diluent**'.
- 3 Tare the balance.
- 4 Use a 1 mL pipette to dispense 1 mL of **BioTek's Calibration Solution** OR the **Start Solution** into the tared tube. The weight should be in the range 0.900 – 1.100 g. Note this value as the '**Weight of Concentrate**'.
- 5 Cap the tube and shake it vigorously.

**Procedure Using Multi-Channel Pipette**

❖ To minimize the effects of evaporation, keep the solution bottle capped when not in use, and close the plate lid immediately after pipetting.

- 1 Create a Gen5 experiment based on the **Pathlength Calibration MultiWell** protocol for the BioCell or cuvette as appropriate.
- 2 Select **Plate > Read**. Within the 'Plate # Reading' dialog:
  - Enter the plate's serial number in the Plate ID field.
  - Enter the '**Weight of Concentrate**'.
  - Enter the '**Weight of Diluent**'.
- 3 Fill the BioCell or cuvette with the 20:1 Solution and place it in the Take3 plate (see p. 14 for proper orientation). If using the BioCell, place it in well A9.
- 4 Pipette 5 µL of Start Solution into all 16 microspots.
- 5 Close the lid gently to avoid splashing, and then place the plate on the carrier.
- 6 In Gen5, click **READ**.
- 7 Click **Continue** when prompted and then click **OK**.

When the read is complete a Curve warning *may* appear if the curve is ambiguous. This is not a problem; click **OK** to close the warning.

- 8 When the read is complete, select **Plate > Print** to print the new Pathlength values (or select **Plate > PowerExport** to view them in Excel).
- 9 In the “Curve Deltas” section of the report, verify that:
  - Std Dev is < 0.002
  - Max Delta (mm) is < 0.0100
 If either or both values are too high, clean the slides (p. 16) and run the experiment again.
- 10 Clean the slides with a dry laboratory wipe (p. 16).
- 11 Update Gen5 with the new Pathlength values (p. 26).

### ***Procedure Using Single-Channel Pipette***

❖ To minimize the effects of evaporation, keep the solution bottle capped when not in use, and close the plate lid immediately after pipetting.

- 1 Create a Gen5 experiment based on the **Pathlength Calibration SingleWell** protocol for the BioCell or cuvette as appropriate.
- 2 Select **Plate > Read**. Within the ‘Plate # Reading’ dialog:
  - Enter the plate’s serial number in the Plate ID field.
  - Enter the ‘Weight of Concentrate’.
  - Enter the ‘Weight of Diluent’.
- 3 Click **READ**.
- 4 Pipette 5  $\mu$ L of Start Solution into microspot A2. Place the plate on the carrier and click **OK, OK**.
- 5 When the read is complete, pipette 5  $\mu$ L of Start Solution into microspot B2, and click **OK**.
- 6 Repeat this process for microspots C2 through H3.
- 7 When prompted, fill the BioCell or cuvette with the 20:1 Solution and place it in the plate (see p. 14 for proper orientation). If using the BioCell, place it in well A9. Click **OK**.

When the read is complete a Curve warning *may* appear if the curve is ambiguous. This is not a problem; click **OK** to close the warning.

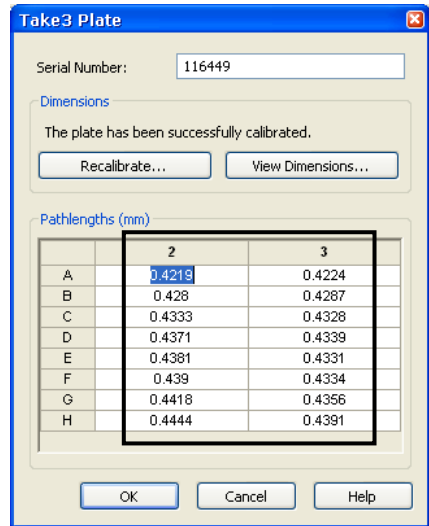
- 8 When the read is complete, select **Plate > Print** to print the new Pathlength values (or select **Plate > PowerExport** to view them in Excel).
- 9 In the “Curve Deltas” section of the report, verify that:
  - Std Dev is < 0.002
  - Max Delta (mm) is < 0.0100

If either or both values are too high, clean the slides (p. 16) and run the experiment again.

- 10 Clean the slides with a dry laboratory wipe (p. 16).
- 11 Update Gen5 with the new Pathlength values (see below).

### Updating Gen5 with New Pathlength Values

- 1 In Gen5, select **Take3 > Take3 Plates**.
- 2 Select the appropriate Take3 plate serial number and click **View/Modify**.
- 3 Replace the 16 existing Pathlengths values with the newly generated values.
- 4 When finished, click **OK**.



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## Specifications

<b>Well Types</b>	16 low-volume microspots, 2 BioCells, 1 cuvette
<b>Sample Volume</b>	Minimum 2 $\mu$ L
<b>Optical Pathlength</b>	0.5 mm (microspots) 1 cm (BioCells and cuvette)
<b>Wavelength range</b>	200 to 999 nm (reader dependent)
<b>Detection Limit</b>	2 ng/ $\mu$ L dsDNA Typical
<b>Absorbance Accuracy*</b>	Epoch Accuracy Specification $\pm 1\% \pm 0.010$ OD (0.0 to 2.0 OD)

\* Absorbance Accuracy is the accuracy specification of the **reader** used for measuring the Take3 plate.



