Take3™ and Take3 Trio™ Micro-Volume Plate

User Guide

BioTek® Instruments, Inc.
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PN 1011000 Rev L

Notices

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Instructions for Use

Should you require instructions for use in a language other than English, please send an email to BioTek Customer Care (CustomerCare@biotek.com) with the plate's serial number and the destination country and desired language. The IFU translation will be provided if the language you requested is an approved language for the destination country for the Professional Use of IVD Devices.

Contents

Contact Information

BioTek® Instruments, Inc.
Highland Park, P.O. Box 998
Winooski, Vermont 05404-0998 USA

Global Service and Support

BioTek instrument service and repair is available worldwide at one of BioTek's International Service Centers and in the field at your location. To arrange for service or repair, contact the office nearest you; visit www.biotek.com for up-to-date contact information. For customer service, sales, and technical assistance, refer to the information below.

Customer Service and Sales

Internet	www.biotek.com
Phone:	888-451-5171 (toll-free in the U.S.) 802-655-4740 (outside the U.S.)
Fax:	802-655-7941
Email:	customercare@biotek.com

Service / Technical Assistance Center (TAC)

Phone:	800-242-4685 (toll-free in the U.S.) 802-655-4740 (outside the U.S.)
Fax:	802-654-0638
Email:	tac@biotek.com

European Coordination Center / Authorized European Representative

BioTek® Instruments GmbH
Kocherwaldstrasse 34
D-74177 Bad Friedrichshall
Germany

Internet	www.biotek.de
Phone:	+49 (0) 7136 9680
Fax:	+49 (0) 7136 968 111
Email:	info@biotek.de

Intended Use Statement

The Take3 and Take3 Trio plates can be used in compatible BioTek detection systems to perform measurements with very low (2 µL) sample volumes. The Take3 and Take3 Trio plates can also be used to measure samples in the BioTek BioCell and/or stoppered cuvettes.

If the instrument has an "IVD" label, it may be used for clinical and non-clinical purposes, including research and development. If there is no such label, the instrument may be used only for research and development or other non-clinical purposes.

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 or by calling (888) 451-5171 or (802) 655-4740.

CE Mark

CE 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 (if labeled for this use)

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.

Warnings, Hazards, and Precautions

When operated in a safe environment according to the instructions in this document, there are no known hazards associated with the Take3 and Take3 Trio plates. The user should be aware of certain situations that could, however, result in injury or damage to the plate. 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! Service. Only qualified technical personnel should perform service procedures on internal components.

Warning! Accessories. Only accessories that meet the manufacturer's specifications shall be used with the instrument.

Warning! Pinch Hazard. When replacing slides, set one short end of the slide into its housing and then lay the slide flat to avoid pinching your finger.

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 or Take3 Trio 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 or Take3 Trio 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 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 from 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 or Take3 Trio 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.

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.

Document Revision History

Rev.	Date	Changes
A	08/2011	First Release
B	10/2011	Added additional optional accessories; moved note about slide cleanliness to the Cleaning section; updated specifications
C	10/2012	Updated second bullet under “Intended Use Statement,” added “(if labeled for this use)” to the heading for Directive 95/79/EC, added “Service” and “Accessories” warnings.
D	3/2014	Added support for Gen5 RC and Gen5 IVD v2.05 and higher; included a note with the part numbers for the calibration solution kits that 4690007 is for use with Gen5 2.0 and higher and 4690008 is for Gen5 1.x and Take3 only; added support for the Synergy Neo, Cytation 3, Synergy 4, and PowerWave XS.
E	1/2015	Added support for the Cytation 5; updated contact information; changed “RMA” to “service authorization number”; added note that it is recommended that the microspot samples not be larger than 2 µL.
F	7/2015	Updated the Contact Information section, changed reference to “service authorization number” to “Service Call Notice” number, clarified the read procedure to determine if a slide is clean, clarified the description of the pathlength calibration procedure, added support for the Synergy Neo2, Cytation 5, Synergy HTX, and Epoch 2, removed support for PowerWave XS.
G	5/2016	Added support for Gen5 v3.x.
H	10/2016	Updated note about recommended volume for samples.
I	6/2017	To add support for Cytation 1, replaced mention of specific Cytation models with just “Cytation.”
J	3/2018	In the Pathlength Calibration method, changed the measurement from 428-530 nm to a simpler 428 nm. Updated instructions for accessing the BioTek CRC to download pathlength data. Added information for instruments with onboard Take3 capability, such as Synergy LX.
K	10/2018	Under Notices, added ‘Instructions for Use’. Under Specifications, clarified that Synergy LX supports Take3 Trio via Gen5 control.
L	3/2019	Added new Appendix A with information about creating custom Protein 280 Sample Types with different versions of Gen5 software.

About Take3 and Take3 Trio Micro-Volume Plates

The BioTek Take3 and Take3 Trio Micro-Volume Plates allow quick and efficient nucleic acid and protein quantification using up to three methods: very low volume (2 µL) sample measurements, BioCell 1 cm measurements, and standard cuvette measurement (Take3 only).

Measurements are made using compatible BioTek absorbance microplate readers and Gen5, which contains pre-programmed applications, including:

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

Gen5 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.

❖ BioTek absorbance readers with onboard Take3 support, such as the Synergy LX with touchscreen, can perform calculations without the need for Gen5. The raw ODs, ratio and calculated concentrations can then be printed or exported. Refer to the instrument's operator's manual for more information.

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

Plate Description

The Take3 and Take3 Trio plates are dimensionally similar to standard 96-well microplates and support the following read types:

	Take 3	Take3 Trio
2 mm 2 µL "microspots" arranged in a 2x8 geometry	16 (wells A2–H3)	48 (wells A2–H3, A5–H6, and A8–H9)
BioCells	Wells A9 and H9	Wells C11 and F11
Cuvette, stoppered with a maximum length of 54 mm	E10 and E11	N/A

Access the microspots by opening the plate lid and exposing the Teflon-coated fused-silica glass slide(s) (**Figure 3** and **Figure 4**).

After pipetting samples, gently close the lid (which also has a glass slide(s)) to form a consistent 0.5 mm pathlength (**Figure 5**).

Figure 1: Take3 plate closed, showing BioCells and cuvette

Figure 2: Take3 Trio plate closed

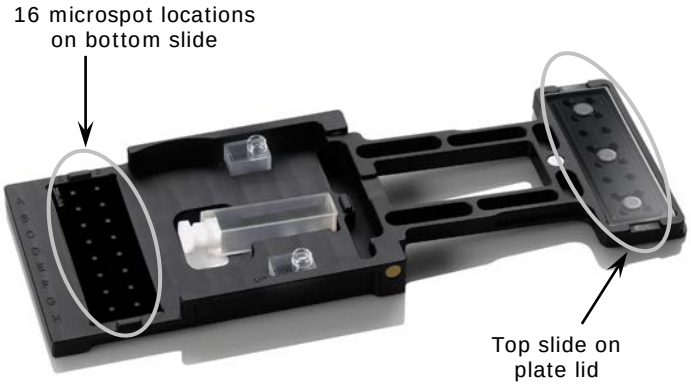


Figure 3: Take3 plate open, showing slides and “microspots”

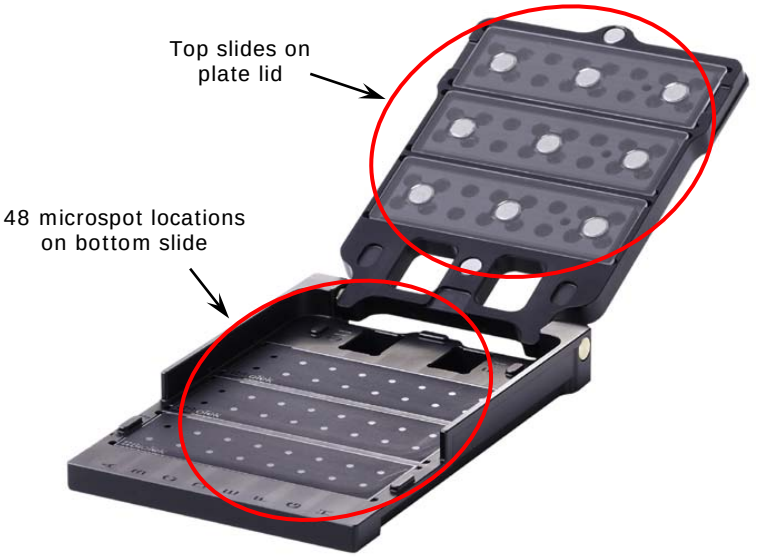


Figure 4: Take3 Trio plate open, showing slides and “microspots”

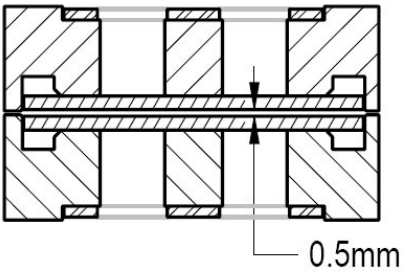


Figure 5: Cross-section of microspots showing top and bottom glass slides with nominal 0.5 mm pathlength

Package Contents

Storage case containing:

- Take3 or Take3 Trio Micro-Volume Plate and user guide
- Data sheet with plate-specific pathlength calibration data
- Two pockets for storing BioTek’s BioCell quartz vessels
- Two pockets for storing cuvettes (not used for Take3 Trio)

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)
- Slide replacement kit with calibration solution (PN 4690010)

❖ Each kit contains one pair of slides. To order replacements for an entire Take3 Trio plate, order three individual kits.

- Calibration solution, 25 mL (PN4693002)
- Calibration solution kit with one BioCell (PN 4690007)(for use with Gen5 v2.0 and above)
- Calibration solution kit with one cuvette (PN 4690008)(for use with Gen5 v1.0 and Take3 module only)
- Microspot printed replacement slide (PN 4690503)
- Blank replacement slide (PN 4690504)

Software Requirements

Take3	Take3 Trio
Gen5 v1.x and higher	Gen5 and Gen5 Secure v2.x and higher Gen5 RC and Gen5 IVD v2.5 and higher
Microsoft Excel is required for results reporting, if the plate is measured using Gen5.	

❖ See Appendix A if you have Gen5 version 3.05 or lower and will create custom Protein 280 Sample types.

Returning the Plate to BioTek

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

1. Contact BioTek TAC (page 4).
2. Decontaminate the plate (page 28).
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: <work order number>
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

❖ These instructions only apply when using Gen5 to configure and measure the plate. If you will use the reader's onboard software, refer to the reader's operator's manual instead.

1: Install Gen5

Measurement collection, data reduction, and results output are performed using BioTek's Gen5 software. Refer to the installation instructions in the *Gen5 Getting Started 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: Download Pathlength Correction Data (optional but recommended)

Each Take3 and Take3 Trio plate is shipped with a data sheet (Take3) or data sheets (Take3 Trio) that contain the pathlength correction data for the microspots of that specific plate. The data for each plate is also available in electronic format for download from the BioTek Customer Resource Center (CRC).

Although you can manually enter the pathlength data in Gen5, it is recommended that you download and import the data into Gen5 to avoid introducing errors.

1 Go to www.biotek.com, Service & Support, Customer Resource Center. Click **Login**.

2 Enter your user ID and password, then click **Login**.

3 Click the **Products** tab. Under 'Products Registered to You' click the link for the serial number of the plate you are using. The data sheets are located under the Files sub-tab at the product detail screen. If the product you're looking for is not listed under Products Registered to You, enter the serial # in the 'Add a Product' section and click submit. This will immediately add that product to your inventory.

❖ If you have questions about how to use the CRC, please contact BioTek Customer Service.

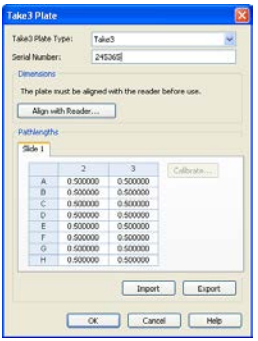
4: Add a Take3 or Take3 Trio Plate to Gen5

Before the plate is used for the **first time**, you must add it to Gen5.

1. Turn on the reader and start Gen5.
2. In the main Gen5 screen, select **Take3** > **Take3 Plates**, and click **New**.
3. Select the Take3 Plate Type.
4. Enter the Take3 plate's **Serial Number**.

Next, you must run the **alignment procedure** with the microplate reader to capture the positions of the plate's microspots.

1. Place the plate on the microplate carrier. Align "A1" on the plate with "A1" on the carrier.
2. Click **Align with Reader**, **Start Alignment**, and **OK**. The process takes several minutes. When finished, click **OK**.



Finally, enter the **pathlength** values associated with your plate's microspots for use in calculating concentrations.

❖ Enter **pathlength** values associated with your plate's microspots for the most accurate results.

1. The Gen5 Pathlengths table for the Take3 plate contains the default 0.5 mm values for 16 locations (A2–H3). For the Take3 Trio, the table contains the default 0.5 mm values for locations A2–H3 (Slide 1), A5–H6 (Slide 2), and A8–H9 (Slide 3).
2. Click **Import** and navigate to where you stored the Pathlength data file for your plate (see **3: Download Pathlength Correction Data (optional but recommended)**)

   OR: Manually replace the default 0.5 mm values with those from the data sheet (included in the plate's storage box).
3. When finished, click **OK**.

5: Set Take3 Plate Preferences

❖ To define Take3 preferences, you must have the **Edit Take3 Settings** permission enabled.

In the Take3 Preferences dialog, you can define the calculation methods and Excel spreadsheet setup for displaying the results of a Take3 session.

Define Calculation Methods

❖ Click **Reset Defaults** to restore the Gen5 default values in the Take3 Preferences dialog.

The Calculation pane enables you to define the blanking method, reference wavelength, spectrum wavelengths, pathlength normalization, and concentration units used in a Take3 session.

Click **Take3 > Preferences**, and select **Calculation** under Nucleic Acid Quantification or Protein A280.

- **Blanking Method**
  - **Blank on each sample well:** Gen5 performs a blank read on each user-selected sample well (microspot, BioCell, or cuvette) on the Take3 plate. The blank value of each well is then subtracted from the sample read of that same well. This option provides high accuracy, particularly when different buffers or samples are being read.
  - **Use blank average:** You must specify one or more locations on the Take3 plate to be read. Gen5 reads the selected blanks, then calculates the average and subtracts that average from each sample measurement. This option provides a greater throughput than blanking on each location and is most appropriate when all samples are read in the same buffer.

    If two or more blanks are read, Gen5 calculates the CV% to be used as a quality assurance check. If the CV% exceeds a user-defined limit (specified in the **Preferences > Calculation** screen), a warning appears, and Gen5 will not perform a sample read until a valid blank read is made. Individual wells can

be included or discarded by clicking on the wells. Gen5 updates the blank statistics each time a well is included or discarded.

- **Reference Wavelength/Limit:** The reference wavelength is used to compensate for the presence of contaminants in the sample. Gen5 subtracts the result of the read at the reference wavelength from the reads at 260 and 280 nm before performing calculations. The default setting is 320 nm.

Gen5 uses the Limit value to validate the sample read value. If the difference between the sample's reference wavelength (raw data) and the blank's reference wavelength (raw data) exceeds the defined limit, all output associated with the affected wells in the sample report and the summary report is displayed with a yellow background and the following message is shown at the bottom of the report: Yellow background indicates a high reference wavelength read.

- **Spectrum wavelengths:** You can define the Start and Stop wavelengths as well as the Step increment for the Take3 read. The default values for Nucleic Acid Quantification are 240 nm/300 nm with a step of 2 nm; Protein A280 default values are 260 nm/320 nm with a step of 2 nm.
- **Normalize Pathlength To:** Gen5 performs pathlength correction to normalize data (blanks and samples) obtained by the reader to the specified pathlength, either 0.5 mm or 1 cm. The pathlength correction is performed on a per-well basis using the calibrated pathlength data associated with the Take3 plate's serial number.
- **Concentration Units:** Select from the list of available units of measure.

Define the Excel Spreadsheet

The results of each sample read from a Take3 session, as well as system information and blanking data, are automatically exported to Excel. You can define how Excel workbooks are created and displayed for new batches and sample reads, and how batch summaries are exported.

1 Click **Take3 > Preferences**, then select **Excel** under either Nucleic Acid Quantification or Protein A280.

2 Under **For new batches**, select an option for how new Take3 batches are displayed:

- **New workbook:** A new workbook is created for the batch.
- **Current workbook:** The current workbook is appended with the new batch's results.
- **Existing workbook:** An existing workbook, which is not currently open, is appended with the new batch's results. You can browse for the existing workbook.

3 Under **For new sample reads**, select an option for how new sample reads are displayed:

- **New worksheet:** Each new read is displayed in its own worksheet.
- **Append to bottom of current worksheet:** The results of each new sample read are appended to the bottom of the current worksheet.
- **Append to right of current worksheet:** The results of each new sample read is appended to the right of the data currently displayed in the current worksheet.

4 Under **For batch summaries**, select an option for how batch summaries are displayed:

- **New worksheet:** Each new batch summary is displayed in its own worksheet.

❖ If you select one of the Append options under **For new sample reads**, you can select an Append option under **For batch summaries**.

- **Append to bottom of current worksheet:** Each new session summary is appended to the bottom of the current worksheet.
- **Append to right of current worksheet:** Each new session summary is appended to the right of existing data in the current worksheet.

- **Include a column for sample names:** A column is added to the summary table in which you can enter the sample ID associated with each sample.

Setting Up a Take3 Session

❖ These instructions only apply when using Gen5. If you will use the reader's onboard software, refer to the reader's operator's manual instead.

❖ Only one Take3 session can be open at a time.

A Take3 session can be started from the Task Manager by clicking **Read Now** and selecting one of the Take3 applications. It is recommended that you set up your Take3 session before pipetting to the Take3 plate to avoid evaporation of the samples.

1: Select an Application

You select the application in the Task Manager, but you can change this selection in the Gen5 main screen. Select one of the following Take3 applications from the File pane, on the left side of the Gen5 main screen:

- **Nucleic Acid Quantification:** This application is performed to determine the concentration of DNA or RNA in a sample. The concentration must be known to achieve optimum performance in protocols involving nucleic acid.
- **Protein A280:** This application assesses the purity of a nucleic acid sample. Performing a read at 280 nm indicates the presence of protein or other contaminants in the sample.

2: Select a Plate

If only one Take3 plate is defined, it is selected automatically. Otherwise, select a plate from the Take3 Plate list.

3: Select a Well Type

Select one of the following well types:

- Microspot
- BioCells
- Cuvette

See **Plate Description** on page 10 for a description of the well types.

4: Select a Sample Type

Select a sample type from the Sample Type list, or create a custom sample type.

- ❖ See the Gen5 Help for more information on creating sample types for Take3 sessions.

5: Enable / Disable Spectrum Scanning

Performing a spectrum scan with a Take3 plate allows you to quickly confirm that your sample contains a sufficient amount of nucleic acid. Select or clear the **Scan** checkbox to activate or disable spectral scanning at runtime. Gen5 executes spectral scanning for all selected wells and ignores empty wells. The results of the scan are exported to Excel in a matrix.

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 from 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.

Microspots

- ❖ 2 µL is the recommended volume for most samples. This volume can be increased to 3–5 µL if the 2 µL results are not satisfactory. A volume lower than 2 µL may result in evaporation, particularly with multiple sample loading. Volumes greater than 7 or 8 µL may lead to cross-contamination, depending on the sample matrix.

You will need:

- A calibrated single- or multi-channel pipette capable of accurately pipetting 2 µL, and 0.1-10 µL 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 (Take3) or 48 (Take3 Trio) samples on the slide (see **Figure 6**).

3 When finished, lower the plate lid gently to avoid splashing the samples.

4 Read the plate immediately (page 20).

5 After reading the plate, clean the slides before pipetting fresh samples (page 25).

Figure 6: Pipetting samples

BioCell

- The Take3 and Take3 Trio plates support up to two BioCells (available from BioTek) in plate locations A9 and H9 (Take3) and C11 and F11 (Take3 Trio).
- 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**).
- Ensure that plate locations E10 and E11 do not contain air bubbles.
- When measuring a low-volume cuvette, use plate location E10.

Reading the Plate

❖ These instructions only apply when using Gen5. If you will use the reader's onboard software, refer to the reader's operator's manual instead.

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

Using the Gen5 Take3 Feature

1 If you have not already done so, prepare a Take3 Session (page 20).

2 Prepare the plate (page 21).

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 session:

- Select the blank wells to be read. Click **Read** and then click **OK**.
- After the blank wells are read, click **Approve** to accept the values. Gen5 exports the blank read data to Excel.

❖ You can also click **Reset** to discard the data from the blank read and reread the blanks.

- Click **Read** to read the sample wells, and then click **OK**. Gen5 exports the read data.
- When finished, click **End of Batch**. Gen5 exports the batch summary results.

Using the Gen5 Microplate Interface

1 From Task Manager, select **Read Now > New**.

2 In the Procedure, set the plate type to **Take3 <your plate's serial number>** or **Take3 Trio <your plate's serial number_Slide *x*>**.

3 Click **Read** and define the reading parameters.

4 Select the wells to be read.

- Take3 plate and Take3 Trio Slide 1, select within A2–H3.
- Take3 Trio Slide 2, select within A5–H6.
- Take3 Trio Slide 3, select within A8–H9.

5 Click **OK** and load the plate to begin the experiment.

6 When the experiment has been run, Gen5 prompts you to save it. Navigate to the location where you want to save the file, enter a file name, and click **Save**.

7 Export your data to Excel, if desired.

8 When finished, review the data and select **File > Save** to save the experiment.

Cleaning

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

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.

❖ After cleaning the slides, we recommend that you read the microspots at 260 nm and 320 nm.

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.)

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 (page 30).

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.

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

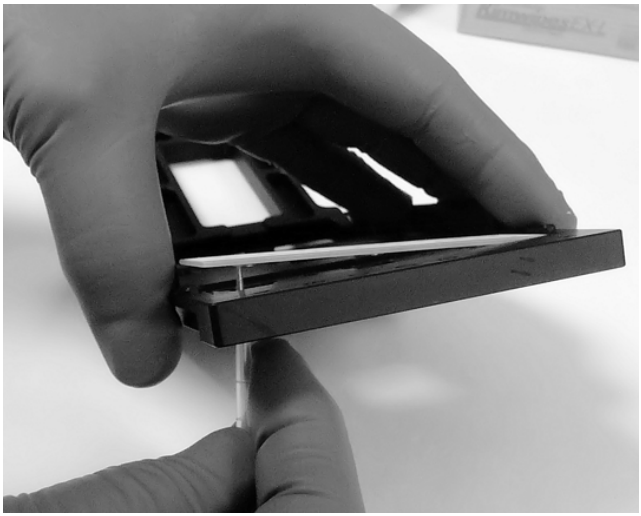


Figure 7: Using a pipette tip to remove a slide

3 (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.

4 Moisten a laboratory wipe with the cleaning solution and wipe the slides on both sides.

5 Rinse the slides in deionized water and dry them with a laboratory wipe.

6 Air-dry the slides or dry them in an incubator (40° to 60° C). If necessary, use clean canned air to remove debris.

7 If necessary, wipe the **plate** with the cleaning solution before replacing the slides:

- Wipe the three contact points (**Figure 8**) 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 8: 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 in **Figure 8**).

9 Before pipetting fresh samples:

- Verify the cleanliness of the slides.

❖ After cleaning the slides, we recommend that you read the microspots at 260 nm and 320 nm.

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.)

- Calibrate the plate pathlengths to ensure the most accurate measurements (page 30).

Decontamination

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

	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. The bleach solution is caustic; wear gloves and eye protection when handling this solution.
	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. Do not prepare a stronger bleach solution than described here. Extended exposure to high concentrations of bleach can deteriorate the plate surfaces. Do not soak the plate or slides in bleach or other harsh cleansers. Do not autoclave the plate or slides.

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 (**Figure 7**).

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 and, for the Take3 Trio, A5 and A8 (refer to **Figure 8**).

5 Before pipetting fresh samples:

- Verify the cleanliness of the slides.
- Calibrate the plate pathlengths to ensure the most accurate measurements.

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

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.)

6 Discard the cleaning materials in an approved biohazard container.

Pathlength Calibration

❖ Gen5 software and Microsoft Excel are required for this procedure.

This section describes procedures for calibrating pathlengths for the microspots when the slides are replaced after cleaning.

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 for details).

Method

The Take3 and Take3 Trio plates are designed with nominal pathlengths of 0.5 mm for their microspots. In reality each microspot has a slightly different pathlength, which can vary from the intended 0.5 mm. During the calibration procedure, the exact pathlength is determined for each microspot and stored in Gen5 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 µL of C1 into each microspot, fill a 1 cm BioCell with C2, and then run a Gen5 experiment to measure the microspots and BioCell at 428 nm. Gen5 performs the pathlength calculations automatically; a brief explanation follows.

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

OD_microspot = K*C1*(pathlength of microspot)
OD2_BioCell = K*C2*(pathlength of BioCell)

From these two equations it follows that:

(pathlength of microspot) = OD_microspot*C2*(pathlength of BioCell)/(OD_BioCell*C1)

or, written another way: (pathlength of microspot) = (OD_microspot/OD_BioCell)*(C2/C1)*10 mm

Materials

- BioTek Calibration Solution Kit with one Biocell (PN 4690007)

❖ You can also purchase the calibration solution (PN 4693002) and the BioCell (PN 7272051) separately.

- 0.5–10 µL multi-channel pipette with 0.1–10 µL pipette tips
- Drummond pipette aid (or equivalent) with 25 mL disposable serological pipette
- 1 mL pipette
- One 50 mL conical tubes with caps
- Disposable pipette troughs
- Lint-free disposable paper wipes
- Beaker or other small container
- Scale readable to 0.0001 g

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** 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.

Pathlength Calibration Procedure

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

1 From the Gen5 main screen, select **Take3 > Take3 Plates**.

2 Highlight the plate you want to calibrate and click **View / Modify**.

3 In the Pathlengths area of the Take3 Plate dialog, click **Calibrate**. For the Take3 Trio, first select the tab for the slide you want to calibrate, then click **Calibrate**. Gen5 performs a blank read to verify the cleanliness of the slide.

When the read is complete, Gen5 displays the data. You can rerun the blank read, continue to the pathlength read, cancel the operation, or search the Help for more information.

❖ We recommend cleaning and verifying the slides again if any OD values are greater than approximately 0.020. (Clean slides *typically* measure less than 0.012 OD.)

4 To accept the blank read data, click **Continue**.

5 Gen5 prompts you to enter:

- Weight of Concentrate
- Weight of Diluent

6 Fill the BioCell with the **20:1 Solution** and place it in well A9 of the Take3 plate or well C11 of the Take3 Trio.

7 Pipette 5 µL of BioTek's Calibration Solution into the appropriate 16 microspots.

8 Close the lid gently to avoid splashing, and then place the plate on the carrier.

9 In Gen5, click **READ**.

10 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.

11 Microsoft Excel opens and displays the pathlength calibration information.

- Gen5 takes all the calculated pathlengths for the 16 spots on each slide and calculates a best fit curve through those 16 data points. It then subtracts each data point from the calculated pathlength from the curve for each spot and reports the absolute value of these differences.
- If any of these differences is larger than 0.010 mm, or if the standard deviation of all the differences is larger than 0.002 mm, then you need to clean the slide(s) and rerun the experiment.

Gen5 automatically populates the plate's pathlength grid with the new values.

❖ If you are using a Take3 Trio, repeat this process for Slides 2 and 3.

12 Clean the slide with a dry laboratory wipe (page 25).

Specifications

	Take3	Take3 Trio
2 µL Sample capacity	16	48
BioCell capacity	2	2
Cuvette capacity	1	N/A
Detection limit	2 ng/µL dsDNA (spectrophotometric)	
Optical pathlength	0.5 mm nominal	
Detection modes	absorbance and fluorescence	
Compatible readers	Cytation, Eon, Epoch, Epoch 2, PowerWave XS2, Synergy 2, Synergy 4, Synergy H1, Synergy H4, Synergy HT, Synergy HTX, Synergy LX, Synergy Mx, Synergy Neo, Synergy Neo2	Cytation, Eon, Epoch, Epoch 2, PowerWave XS2, Synergy H1, Synergy H4, Synergy HTX, Synergy Neo, Synergy Neo2, Synergy LX, via Gen5™ control
Applications	• Micro-volume nucleic acid and protein quantification • In-situ fluorometric protein quantification • 260/280 and 260/230 protein purity measurements • Very low volume in-situ BCA assay • Spectral scan of single or multiple low volume samples	
Use and care	Take3 plates' sample surfaces are easily cleaned with laboratory wipes.	

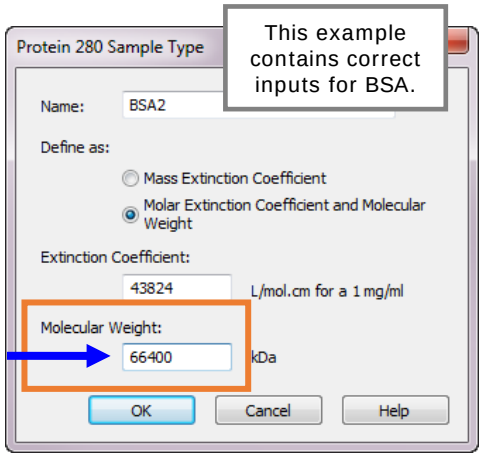
Appendix A: Take3/Take3 Trio—Custom Protein 280 Sample Types

This appendix applies to Take3/Take3 Trio users with Gen5 software version 3.05 or lower, who will create custom Protein 280 Sample Types utilizing the *Molar Extinction Coefficient and Molecular Weight* option.

Disregard this appendix if any of these are true:

- You will use Gen5 software version 3.06 or higher.
- You will use only predefined Protein 280 Sample Types.
- You will use only *Mass Extinction Coefficient* in your custom Protein 280 Sample Types.

Gen5 provides a few predefined Protein 280 Sample Types, including BSA, IgG, Lysozyme, 1 Abs at 1 cm = 1 mg/mL.



Gen5 also provides the option to create custom sample types, by selecting **Take3 > Protein 280 Sample Types** and clicking **New**. If your application requires the new sample type to use the "Molar Extinction Coefficient and Molecular Weight" option, the Molecular Weight unit of measure is *incorrectly* labeled as **kDa** (kilodaltons), as shown above. Enter the Molecular Weight value in **Da** (daltons).

With Gen5 versions 3.06 and higher, this dialog will correctly label the Molecular Weight unit of measure as **Da**.

For more information on this issue, contact BioTek: TAC@biotek.com or (800) 242-4685/(802) 655-4740.