Manual for Procedures and Kit Description

For

Determination of Beryllium Particulates (BeFinder®)*

Portable System

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Compatible with ASTM* Methods D7202, D7458, NIOSH Methods 7704 and 9110**

Questions and Support: 520 321 7680, ext 29

Technology protected by US Patents: 8,003,394; 8450,117; 8,945,931; 9,217,711 & Pending Patents

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* American Society of Testing Materials (ASTM) International (www.astm.org)

**National Institute of Occupational Safety and Health (NIOSH) (www.cdc.gov/niosh)

Table of Contents

I. General Instructions and Guidelines

BeFinder® is a method for detecting particulates of beryllium and its compounds by optical fluorescence. This methodology does not require expensive set-up and can yield results within an hour. The method entails wiping the surface suspected of having beryllium contamination with a wipe, dissolving the beryllium on the wipe and then testing a small fraction of this solution by fluorescence when combined with a dye. The remaining solution may be used for future reference or for confirmation by an alternative method. The method can also be applied to air samples. *Please See NIOSH methods 7704 and 9110 along with the Back-up Data Report (also from NIOSH) and publications (see General Reference section) to understand the technology, validity of the claims and limitations of the system.*

Berylliant provides all the supplies as categorized in the following kits.

Table 1

Kit Description (BeFinder®)	Kit Designation	Number of Wipes/Filters	Summary	More Details
Chemical Kit	CH-A	96	500ml of dissolution solution (CH-1) and 220ml of detection solution (HS-3)	Appendix 5
Consumables Kit	CO-A	96	Consumables to wipe surface and process sample to obtain solution for Be detection	Appendix 3
Processing Kit	PR-A	Indefinite	Hardware needed to process wipes to obtain solution for Be detection	Appendix 6
Fluorometer	FI-B	Indefinite	Trilogy Laboratory Fluorometer and Optical Kit FM-B	Appendix 2
Calibration Standards	ST-A	Up to expiry date	Each of the 5 standards comes with 100ml of calibration solution	Appendix 1 and 4
Consumables Kit for calibration solution preparation	CC-A	Will make up to 100 sets of calibration solutions	Each set of calibration solution may be used for up to two days when stored properly	Appendix 4

Set-up of the sample processing kit is described in Section II. Preparation of solutions of calibration standards is given in Appendix 1. The detailed procedure is in Section III, which describes how and where to use various items in the kits. The fluorometer kit is composed of a Turner Designs Trilogy Laboratory Fluorometer (FI-B) and an optical kit (FM-B). The fluorometer is UL listed and details on the use of this instrument and calibration are in Appendix 2.

Section IV provides a method summary for experienced users.

II. Setting Up Equipment

This procedure describes the set-up of the fluorometer and the various items for processing samples (**Processing kit PR-A, part numbers begin with "PR"**), as shown in **Appendix 6 on page 31**. Please refer to this Appendix and the packaging list included in the sample processing kit as you set up the items. The pictures shown in Appendix 6 are not to scale. Details of assembly of individual items may be found in the OEM instruction books included with each item. Part numbers beginning with "**CO**" are from consumables kit, as shown in **Appendix 3, page 28**.

1. Assemble the stand and the bottle top for water dispenser (Item PR-1). Either glass or plastic bottles may be used. Fill the bottle with de-ionized (DI) water and screw in the assembled top. Prime the pump to ensure that water is being dispensed consistently. Set the dispenser to deliver 0.2 ml of DI water. Verify, by weighing the volume, that exactly 0.2 ml of DI water is being delivered.
2. Assemble the two identical plastic stands (PR-3 and PR-5), which will hold the dissolution solution tubes.
3. Assemble the plastic stand (PR-6) to hold the cuvettes.
4. Assemble the heating blocks with thermometers in the heating unit. Set the temperature to 85°C for dissolution of the beryllium on the media.
5. Open the box for the pipetter stand (PR-9) and place it on the table.
6. Open the box for the 5ml pipetter (PR-2) and adjust the volume to 5ml. Box PR-12, for holding pipette tips (CO-10), is located within this package. Once the tips are consumed, the box should be reloaded with refill tip cartridges supplied in bags. Place the pipette on the stand (PR-9). Check the accuracy of the dispensed volume (5ml) using DI water (use tips CO-10) and weighing this on an analytical balance.
7. Open the box for the 2.5 ml pipetter (PR-7). Take the pipetter out and adjust the volume with the rotary knob to 1.900 ml. Box PR-11, for holding pipette tips (CO-7), is located within this package. Once the tips are consumed, the box is reloaded with refill tip cartridges (CO-7). Check the accuracy of the dispensed volume of 1.9ml

using DI water and weighing this on an analytical balance. Place the pipetter on the pipette stand (PR-9).

8. Open the box for the 0.1 ml pipetter (PR-8) and the pipette tip box (PR-10). The pipette tip box comes loaded with 96 pipette tips. Once the tips are consumed, the box is reloaded with refill tip cartridges (CO-8). Check the accuracy of the dispensed volume of 0.1ml using DI water and weighing this on an analytical balance. Place the pipetter on the pipette stand (PR-9). All the dispensers and the pipetters should be periodically checked for accuracy and adjusted if required, e.g., a recommended period is every 3 months.

A Turner Designs Trilogy Laboratory Fluorometer (FI-B) is provided by Berylliant Inc. This is supplied with an optical kit (FM-B) with an excitation filter at 365nm ± 15nm and emission filter at 480nm ± 5nm. The fluorometer is UL listed.

Any fluorometer with high sensitivity and a wide dynamic range, with proper optical filters, may be used for the fluorescence reading. Preferred excitation wavelengths are between 360 and 380nm (most preferred 365nm) and the preferred emission readout should be in wavelengths of 440 to 490nm (most preferred 475nm).

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III. Detailed Beryllium Test Procedure

Use appropriate hygiene, personal protection and waste disposal methods commensurate with the chemicals. Safety eye protection and gloves must be used when handling solutions. Efficient dissolution of beryllium in unknown samples is highly dependent on their physical and chemical nature. Some experimentation may be required to ensure that good dissolution is being achieved. Correlation with previous/alternative methods is strongly recommended.

The quantification level from this method depends on the fluorometer used, but it is designed to quantify from 0.005µg or above in the media. Limit of detection for the method has been established at 0.0008µg.

The procedure described here uses a Turner Designs Trilogy Laboratory Fluorometer. More details on the fluorometer are in Section II.

Various kits are listed in Table 1 and their contents are identified as below (the pictures in these appendices are not to scale):

- Consumables (CO-1 to CO-10 on **page 28, Appendix 3)**
- Calibration standards (ST-A on p**age 29, Appendix 4**)
- Calibration associated consumables (CC-1 to CC-4 on **page 29, Appendix 4**)
- Chemicals (CH-1 and HS-3 on **page 30, Appendix 5**) and
- Sample processing items (PR-1 to PR-12 on **page 31, Appendix 6**).

1. **Wipe Collection**
    a. Wet filter paper (CO-1) with 0.2 ml DI water using dispenser (PR-1) and wipe a 100cm^2 area. It is recommended that wiping is done according to ASTM method D6966. The Stencil (CO-2) (see Figure 1) may be used as a guide to visualize a 10 cm x 10 cm area. Acceptable wipes are cellulosic filters, such as Whatman filters 541.
    b. Place the wipe in the dissolution tube (CO-3 with blue caps), push it inside, cap and label the tube.
    c. Collect as many samples as desired, each in a separate dissolution tube.

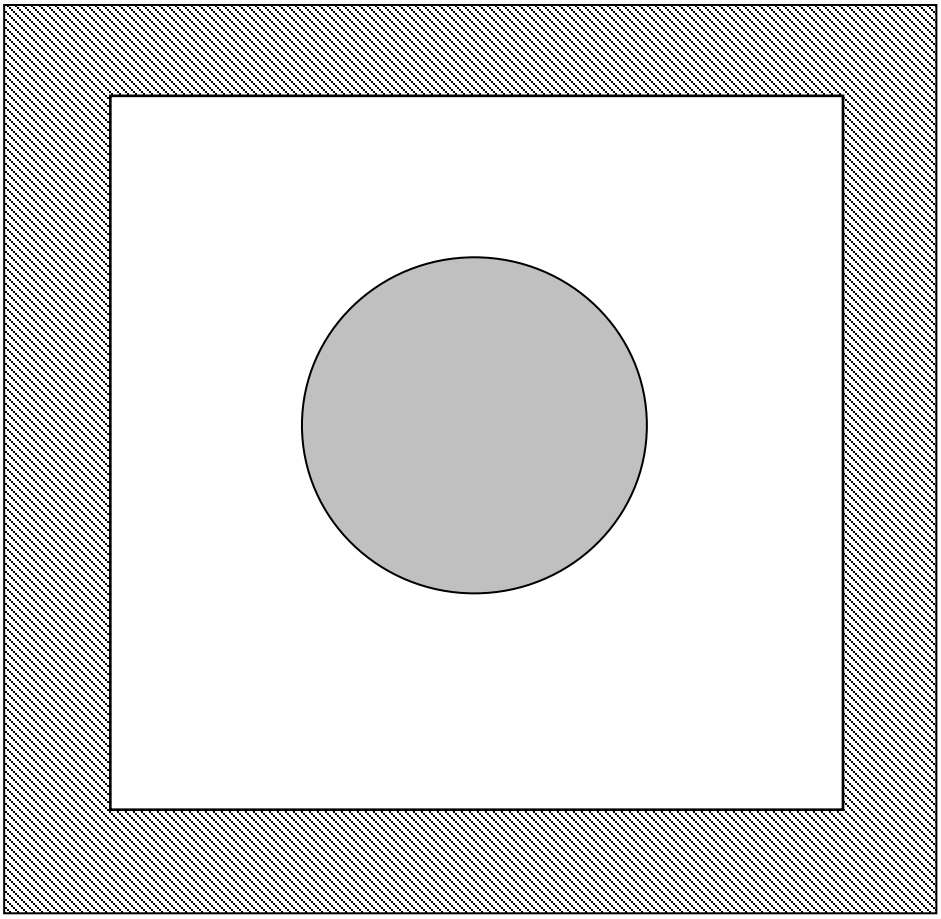

Figure 1: This shows the square stencil (CO-2) to visualize the 10 cm x10 cm wipe area. The round filter paper (CO-1, 47mm diameter) to wipe the surface is also shown to demonstrate its relative size to the 10 cm x10 cm

2. Dissolution Procedure

a. Using the 5ml pipette (PR-2) and tips (CO-10), dispense 5 ml of dissolution solution (CH-1) into each dissolution tube containing a wipe for analysis. Be careful to not touch the tip of the dispenser to the wipe in the tube or to the tube.

b. Repeat step 2a for each sample.

c. The dissolution tubes may be temporarily stored in a stand (PR-3).

d. Place the tubes in the heating block at 85°C for 30 minutes. The solutions must be cooled to room temperature before proceeding further with analysis.

e. Place the dissolution tubes in the stand for temporary storage (PR-3).

3. Dye addition and measurement procedure

a. Remove the syringe plunger from the syringe (CO-4). Attach a luer-lock syringe filter (CO-5) and pour the liquid contents of one dissolution tube into the syringe (do not use any metal needles to draw the fluids into the syringe). Place the plunger back into the syringe and slowly squeeze the liquid contents through the filter into a labeled analyte tube (CO-6 with orange caps). Place analyte tube with filtered solution in another rack (PR-5). Repeat this for all tubes with dissolution solution. These are called the analyte solutions.

b. Take the 1.9 ml pipetter (PR-7) and attach a pipette tip (CO-7 contained in box PR-11). Pipette 1.9 ml of detector solution (HS-3) into an empty, labeled cuvette (CO-9). Fill enough empty cuvettes for your analyte solutions. The same pipette tip (CO-7) may be used for all of the empty cuvettes. Next, take the 0.1 ml pipetter (PR-8) and attach a pipette tip (CO-8 contained in box PR-10). Pipette 0.1 ml of analyte solution (i.e., filtered solution with dissoluted sample) into a labeled cuvette (CO-9) containing 1.9 ml of detector solution (HS-3). Do this for each of the analyte solutions using a new tip (CO-8, contained in box PR-10) for each one to avoid contamination. Cuvettes suitable for fluorescence work should be used. The cuvettes may be put in the cuvette stand (PR-6). Cap the cuvette and mix well by placing a thumb on the bottom of the cuvette and an index finger on the cap of the cuvette and inverting the cuvette several times. Repeat this process for all samples using separate cuvettes. The solution must be clear and colorless. A yellow-gold color shows the presence of iron. These yellow solutions should be left standing for two hours and then re-filtered to remove precipitated iron. The second filtration, if necessary, should be carried out using a PALL 0.2µm GHP ACRODISC Luer-lok™ syringe filter and should be filtered into a new cuvette. Mixing 0.1ml of dissolution solution and 1.9 ml of the detector solution is called 20X dilution. This allows beryllium to be quantified from

0.05µg on the media. On the other hand, use of 5X dilution (see Appendix 7) allows quantification from 0.005µg on the media.

4. **Calibrate the instrument** *(See Appendix 1* ***(Procedure to Prepare Calibration Standards)*** *and Appendix 2 (****Procedure for (1) Using the Turner Designs Trilogy Laboratory Fluorometer and (2) Measuring Beryllium Standards and Storing Calibration Curves****). See Appendix 7 if beryllium quantification is desired from 0.005µg on the media.*

5. **Measurement of collected samples**

   a. The calibration curve obtained, using the prepared standards 0, 0.5, 2.0, 10.0 and 40.0 ppb, is used for reading the collected samples.

   b. Insert a cuvette with collected (unknown) sample in the sample compartment, close the lid and touch "Measure Fluorescence". The instrument gives a reading in ppb.

   c. **Do not store samples in the sample chamber of the instrument as their temperature may change resulting in erroneous readings.**

   d. To convert the data from ppb reading on the instrument to µg/ wipe, divide the ppb reading by 10. For example, a reading of 10 ppb on the instrument corresponds to 1 µg/100 cm^2, assuming that a 100 cm^2 area was wiped. Results from wiped areas less than or greater than 100 cm^2 must be normalized. Similarly, the results from the air filter need to be normalized to 1,000 liters ($1m^3$) of air passed through the filter.

   e. **It is recommended that the instrument be recalibrated after each time it is shut off or at least twice a day. More frequent calibration may be required if it is suspected that the temperature of the environment has changed by more than 3°C.** The standard calibration solutions in cuvettes must be stored in the provided amber colored jar when not in use. This will prolong their life so that they may be used for up to two days.

Measurement of collected wipe samples with elevated overlapping background fluorescence.

In some cases, wipe samples can give a slightly high false positive beryllium content due to an elevated fluorescence signal coming from specific organic impurities with overlapping fluorescence signal. These impurities can be safely removed using a BeFinder specially treated carbon black (BeFinder CB-1). Before using the BeFinder CB-1 treatment, one can determine the presence of such impurities by placing the sample fluid (ABF after sample dissolution), without any dye addition, in a cuvette and measuring its fluorescence. This should read below 0.04ppb. If not, then there are overlapping organic fluorescence impurities present which must be removed by carrying out the dissolution of the sample in ABF along with the BeFinder CB-1, as described below, specifically in section 7.

6. Wipe Collection

a. Wet filter paper (CO-1) with 0.2 ml DI water using dispenser (PR-1) and wipe a 100cm^2 area. It is recommended that wiping is done according to ASTM method D6966. The Stencil CO-2 (see Figure 1) may be used as a guide to visualize a 10 cm x 10 cm area. Acceptable wipes are cellulosic filters, such as Whatman filters 541.

b. Place the wipe in the dissolution tube (CO-3 with blue caps), push it inside, cap and label the tube.

c. Collect as many samples as desired, each in a separate dissolution tube.

7. Dissolution Procedure Modified with BeFinder CB-1

a. **To a 500ml bottle of dissolution solution (CH-1) add 0.5g of BeFinder CB-1. Shake well to form a dispersion**.

b. **Using the 5ml pipette (PR-2) and tips (CO-10), dispense 5 ml of dissolution solution (CH-1 + BeFinder CB-1) into each dissolution tube containing a wipe for analysis. Be careful to not touch the tip of the dispenser to the wipe in the tube or to the tube.**

c. Repeat step 7b for each sample.

d. The dissolution tubes may be temporarily stored in a stand (PR-3).

e. Place the tubes in the heating block and at 85°C for 30 minutes. The solutions must be cooled to room temperature before proceeding further with analysis.

f. Place the dissolution tubes in the stand for temporary storage (PR-3).

If a set of samples have been treated in 5.0 ml batches of ABF and then found to have overlapping fluorescence signals. The organic impurities can be removed by addition of BeFinder CB-1 to each falcon tube in a concentration of 5mg and then repeating the steps "e" to "f" as described above.

8. Dye addition and measurement procedure

a. Remove the syringe plunger from the syringe (CO-4). Attach a luer-lock syringe filter (CO-5) and pour the liquid contents of one dissolution tube into the syringe (do not use any metal needles to draw the fluids into the syringe). Place the plunger back into the syringe and slowly squeeze the liquid contents through the filter into a labeled analyte tube (CO-6 with orange caps). Place the analyte tube with filtered solution in another rack (PR-5). Repeat this for all tubes with dissolution solution. These are called the analyte solutions.

b. Take the 1.9ml pipetter (PR-7) and attach a pipette tip (CO-7 contained in box PR-11). Pipette 1.9ml of detector solution (HS-3) into an empty, labeled cuvette (CO-9). Fill enough empty cuvettes for your analyte solutions. The same pipette tip (CO-7) may be used for all of the empty cuvettes. Next, take the 0.1 ml pipetter (PR-8) and attach tip (CO-8 contained in box PR-10). Pipette 0.1 ml of analyte solution (i.e., filtered solution with dissoluted sample) into a labeled cuvette (CO-9) containing detector solution (HS-3). Do this for each of the analyte solutions using a new tip (CO-8 contained in box PR-10) for each one to avoid contamination. Cuvettes suitable for fluorescence work should be used. The cuvettes may be put in the cuvette stand (PR-6). Cap the cuvette and mix well by placing a thumb on the bottom of the cuvette and an index finger on the cap of the cuvette and inverting the cuvette several times. Repeat this process for all samples using separate cuvettes. The solution must be clear and colorless. A yellow-gold color shows the presence of iron. These yellow solutions should be left standing for two hours and then re-filtered to remove precipitated iron. The second filtration, if

necessary, should be carried out using a PALL 0.2µm GHP ACRODISC Luer-lok™ syringe filter and should be filtered into a new cuvette. Mixing 0.1ml of dissolution solution with 1.9 ml of the detector solution is called 20X dilution. This allows beryllium to be quantified from 0.05µg on the media. On the other hand, use of 5X dilution (see Appendix 7) allows quantification from 0.005µg on the media.

9. **Measurement of collected samples as described in section 5 above.**

IV. Summary of Beryllium Test Procedure

Use appropriate hygiene, personal protection and waste disposal methods commensurate with the chemicals. Safety eye protection and gloves must be used when handling solutions. Efficient dissolution of beryllium in unknown samples is highly dependent on their physical and chemical nature. Some experimentation may be required to ensure that good dissolution is being achieved. Prior to application of the Berylliant method, the user should review the detailed procedure in Section III.

Various consumables, standards and associated consumables, chemicals and sample processing equipment are provided in kits, as identified in Appendix 3, 4, 5 and 6 respectively and Table 1. The quantification level from this method depends on the fluorometer used, but the method is designed to quantify to 0.005µg or above on the media.

1. Wet filter paper with 0.2 ml of water and wipe a surface area of 100cm^2 suspected of Be contamination. Whatman 541 filter paper may be used as a wipe. If air samples need to be analyzed, mixed cellulose ester (MCE) filters are recommended. For analysis of soil samples see ASTM D7458 method.
2. Place the wipe or the filter in a dissolution tube (with blue cap) and add 5ml of dissolution solution.
3. Prepare up to 32 samples by repeating steps 1 and 2.
4. Place the dissolution tubes in the heating block at 85°C for 30 minutes. The solutions must be cooled to room temperature before proceeding further with analysis.
5. After heating, filter the dissolution solution into new tubes (with orange cap). Filtration is done using individual syringes along with the Luer-Lok™ filters.
6. Samples for fluorescence measurement are prepared by adding 0.1 ml of dissolution solution to 1.9 ml of the detection solution (called 20X dilution). Remaining dissolution solution may be used for other analysis or repeating this analysis later if needed. Using 20X dilution, beryllium may be quantified from 0.05µg on the media. Use of 5X dilution (see Appendix 7) allows quantification from 0.005µg.
7. Calibrate the fluorometer using 0, 0.5, 2, 10 and 40 ppb standards prepared by adding 0.1 ml of standards (0, 10, 40, 200 and 800 ppb) to 1.9 ml of the detection solution (20X dilution). These prepared calibration solutions may be re-used up to two days if

they are stored in capped bottles or cuvettes and away from light below 450 nm wavelength without any evaporation losses. Preferably, the standards should be prepared on the day of the analysis and kept in capped cuvettes.

Measure fluorescence from the samples and convert it to beryllium quantity in micro grams (μg) from 100 cm^2 of wiped area. Air filter samples should be normalized to a flow volume of 1,000 liters ($1m^3$) through the filter. Correlation between beryllium content from fluorescent solutions and beryllium content on the wipe or filter is shown in Table 2.

V. General References Related to the Method

1. ASTM Test Method D7202-06 Standard Test Method for Determination of Beryllium in the Workplace Using Field-Based Extraction and Fluorescence Detection http://webstore.ansi.org/ansidocstore/product.asp?sku=ASTM+D7202%2D06

2. NIOSH Test Method 7704, NIOSH Manual of Analytical Methods (NMAM), 5th Edition, 2007, http://www.cdc.gov/niosh/nmam/ .

3. NIOSH Test Method 9110, NIOSH Manual of Analytical Methods (NMAM), 5th Edition, 2007, http://www.cdc.gov/niosh/nmam/ .

4. Minogue E.M, Ehler DS, Burrell AK, McClesky TM, Taylor TP. Development of a new fluorescence method for the detection of beryllium on surfaces. J. ASTM Int., **2(9)**, 10pp. Paper ID JA113161 (2005)

5. Ashley K, Agrawal A, Cronin J, Tonazzi J, McCleskey TM [2005], Backup data-Method nos. 7704 and 9110/ Beryllium Issue 1, NIOSH Docket Office, Mailstop C-34, 4676 Columbia Parkway, Cincinnati, OH 45226, email NIOSHDOCKET@cdc.gov .

6. Agrawal, Anoop; Cronin, John; Agrawal, Akshay; Tonazzi, Juan Carlos; Adams, Lori; Ashley, Kevin; Brisson, Michael; Duran, Brandy; Whitney, Gary; Burrell, Anthony; McCleskey, T. Mark; Robbins, James; White, Kenneth, *Extraction and Optical Fluorescence Method for the Measurement of Trace Beryllium in Soils*, Journal of Environmental Science & Technology, **42(6)**: 2066-2071 (2008).

7. Cronin, J.; Agrawal, A.; Adams, L.; Tonazzi, J.; Brisson, M.;White, K.; Marlow, D.; Ashley, K. Interlaboratory evaluation of an extraction and fluorescence method for the determination of trace beryllium in soils. Journal of Environmental Monitoring, **10**, 955 - 960 (2008) DOI: 10.1039/b804313b.

8. Kevin Ashley, T. Mark McCleskey, Michael Brisson, Gordon Goodyear, John Cronin and Anoop Agrawal, *Interlaboratory Evaluation of a Portable Fluorescence Method for the Measurement of Trace Beryllium in the Workplace*, Journal of ASTM International, Vol 2 (9), paper ID JAI13156 (2005).

9. Anoop Agrawal, John Cronin, Juan Tonazzi, T. Mark McCleskey, Deborah S. Ehler, Edel M. Minogue, Gary Whitney, Christopher Brink, Anthony K., Burrell, Benjamin Warner, Michael J. Goldcamp, Paul C. Schlect, Prerna Sonthalia and Kevin Ashley, *Validation of a Portable Fluorescence Method for the Measurement*

of Trace Beryllium in the Workplace Air and Wipe Samples, Journal of Environmental Monitoring, vol 8: 619-624 (2006)

10. Ashley K, Agrawal A, Cronin J, Tonazzi J, McCleskey TM, Burrell AK and Ehler DS: Ultra-trace determination of beryllium in occupational hygiene samples by ammonium bifluoride extraction and fluorescence detection using hydroxybenzoquinoline sulfonate. *Anal. Chim. Acta*, vol 584: 281-286 (2007)

11. AIHA, Application for Laboratory Accreditation, Effective Nov 8, 2006 (http://www.aiha.org/Content/LQAP/documents) http://www.aiha.org/1documents/lab/lqapnews1006.pdf

12. Occupational Safety and Health Administration (OSHA) Website; http:/osha.gov/SLTC/beryllium/index.html

Appendix 1: Procedure to Prepare Calibration Standards 20X Dilution, (see Appendix 7 for 5X dilution)

Materials and equipment required:

1. Calibration Standards (0, 10, 40, 200 and 800 ppb Beryllium) (See table below)
2. Berylliant detection solution (**HS-3, page 30**)
3. 2 ml cuvettes, suitable for fluorescence work, with caps (**CC-4, page 29**)
4. Dispensing pipetters 0.1 ml fixed volume (**PR-8, page 31**) and 0.5 to 2.5 ml variable volume (**PR-7 page 31**) and corresponding tips **CC-3 and CC-2 (page 29)**.

The 10 ppb standard is most prone for change with time due to the low concentration of Be. Please replace all standards if any standard has started to drift by more than 10% or when the standards expire (whichever comes first).

Procedure

1. Take the 1.9ml pipetter (PR-7) and attach tip (CC-2, same part as CO-7). In each of 5 different cuvettes (CC-4), pipette 1.9 ml of the detection solution (HS-3). Take the 0.1ml pipetter (PR-8) and attach tip (CC-3, same part as CO-8). Dispense 0.1 ml of 0 ppb Be standard solution into one of the cuvettes, cap it, and label the cuvette. Mix well by placing a thumb on the bottom of the cuvette and an index finger on the cap of the cuvette and inverting the cuvette several times. Repeat this with the other four Be standards (10, 40, 200 and 800 ppb) using a new pipette tip (CC-3) each time. The final concentrations of Be in the cuvettes are now 0, 0.5, 2, 10 and 40 ppb. See Table 2 for the summary of standard solutions.
2. Store these calibration solutions in the amber bottle (CC-1) until used for calibration.

Calibration standards and ordering information (**ST-A, page 29**)

These are all Custom Claritas Standards (PPT Grade) with six-month expiration
Matrix: 1% Ammonium bifluoride/H_2O/trace HNO_3

Standard	Amount of Beryllium, ppB	Quantity (ml)
ZENKIAZ-3-100/01	0	100
ZENKIAZ-4-100/01	10	100
ZENKIAZ-5-100/01	40	100
ZENKIAZ-6-100/01	200	100
ZENKIAZ-7-100/01	800	100

Table 2: Summary of "Solution Standards" for Calibration

Preparation of Standard Solutions	**Concentration of beryllium (ppb) in cuvettes comprising calibration standards and detector solution**	**Comments**
0.1 ml of **0 ppb standard** + 1.9 ml of detection solution	0.0	Corresponds to 0.00 µg Be per wipe/air filter
0.1 ml of **10 ppb standard** + 1.9 ml of detection solution	0.5	Corresponds to 0.05 µg Be per wipe/air filter
0.1 ml of **40 ppb standard** + 1.9 ml of detection solution	2.0	Corresponds to 0.2 µg Be per wipe/air filter
0.1 ml of **200 ppb standard** + 1.9 ml of detection solution	10.0	Corresponds to 1 µg Be per wipe/air filter
0.1 ml of **800 ppb standard** + 1.9 ml of detection solution	40.0	Corresponds to 4 µg Be per wipe/air filter

Appendix 2:
Procedure for (1) Using **Turner Designs Trilogy Laboratory Fluorometer** (2) Measuring Beryllium Standards and Storing Calibration Curves.

Figure 2: Turner Designs Trilogy Laboratory Fluorometer

FI-B

The Fluorometer is UL listed

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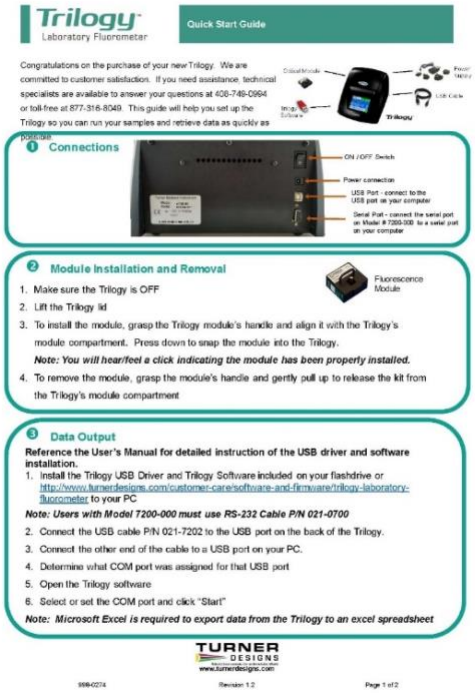
For instructions regarding the Fluorometer, please see the operating quick start guide supplied with the instrument, a copy of which is shown below. Follow instructions for operating fluorometer.



Trilogy Laboratory Fluorometer — Quick Start Guide

Congratulations on the purchase of your new Trilogy. We are committed to customer satisfaction. If you need assistance, technical specialists are available to answer your questions at 408-749-0994 or toll-free at 877-316-8049. This guide will help you set up the Trilogy so you can run your samples and retrieve data as quickly as possible.

1 Connections

ON / OFF Switch
Power connection
USB Port - connect to the USB port on your computer
Serial Port - connect the serial port on Model # 7200-000 to a serial port on your computer

2 Module Installation and Removal

Fluorescence Module

1. Make sure the Trilogy is OFF
2. Lift the Trilogy lid
3. To install the module, grasp the Trilogy module's handle and align it with the Trilogy's module compartment. Press down to snap the module into the Trilogy.
   Note: You will hear/feel a click indicating the module has been properly installed.
4. To remove the module, grasp the module's handle and gently pull up to release the kit from the Trilogy's module compartment

3 Data Output

Reference the User's Manual for detailed instruction of the USB driver and software installation.

1. Install the Trilogy USB Driver and Trilogy Software included on your flashdrive or http://www.turnerdesigns.com/customer-care/software-and-firmware/trilogy-laboratory-fluorometer to your PC

Note: Users with Model 7200-000 must use RS-232 Cable P/N 021-0700

2. Connect the USB cable P/N 021-7202 to the USB port on the back of the Trilogy.
3. Connect the other end of the cable to a USB port on your PC.
4. Determine what COM port was assigned for that USB port
5. Open the Trilogy software
6. Select or set the COM port and click "Start"

Note: Microsoft Excel is required to export data from the Trilogy to an excel spreadsheet

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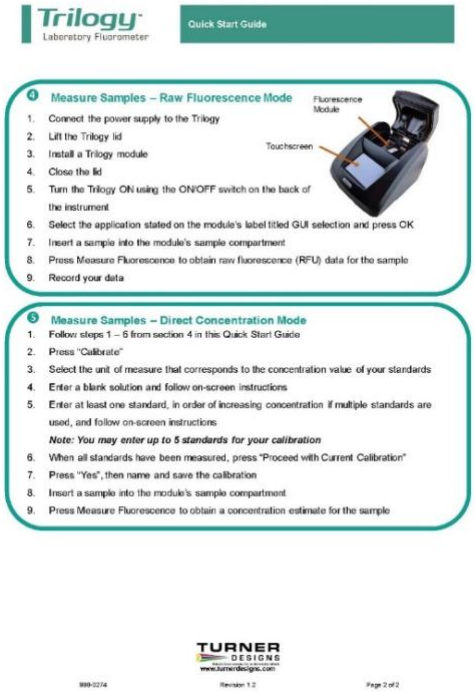

Trilogy Laboratory Fluorometer — Quick Start Guide

4 Measure Samples – Raw Fluorescence Mode

Fluorescence Module
Touchscreen

1. Connect the power supply to the Trilogy
2. Lift the Trilogy lid
3. Install a Trilogy module
4. Close the lid
5. Turn the Trilogy ON using the ON/OFF switch on the back of the instrument
6. Select the application stated on the module's label titled GUI selection and press OK
7. Insert a sample into the module's sample compartment
8. Press Measure Fluorescence to obtain raw fluorescence (RFU) data for the sample
9. Record your data

5 Measure Samples – Direct Concentration Mode

1. Follow steps 1 – 6 from section 4 in this Quick Start Guide
2. Press "Calibrate"
3. Select the unit of measure that corresponds to the concentration value of your standards
4. Enter a blank solution and follow on-screen instructions
5. Enter at least one standard, in order of increasing concentration if multiple standards are used, and follow on-screen instructions
   Note: You may enter up to 5 standards for your calibration
6. When all standards have been measured, press "Proceed with Current Calibration"
7. Press "Yes", then name and save the calibration
8. Insert a sample into the module's sample compartment
9. Press Measure Fluorescence to obtain a concentration estimate for the sample

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(1) Connections

1. Connect the power supply to the Turner Designs Trilogy Laboratory Fluorometer (FI-B).
2. Install the custom Fluorescence Optical Kit (FM-B).
3. Turn on the Fluorometer (switch at the back of the unit).
4. Connect the Fluorometer to a PC using either the UBS cable or the 9 pin serial cable (optional).
5. Install the Trilogy Software on a PC to import data from the Fluorometer to an Excel spreadsheet as follows:

Installation Instructions

This procedure will install the Trilogy software and replace the original spreadsheet with the Berylliant spreadsheet.
Connect the unit to your computer using either the USB cable or the RS232 cable.

Trilogy Software and Berylliant Spreadsheet Installation Instructions:

Before installing the software, open the top of the fluorometer and install the custom optics component by pressing it firmly in place until a click is heard and then turning on the fluorometer.

1) Insert the Turner Designs flash drive into a USB drive on your computer
2) Open the "Environmental Instrument Documentation" folder
3) Open the "Trilogy" folder
4) Open the "Trilogy Software (005-7201)" folder
5) Click on "Setup" and follow the onscreen instructions to install the Trilogy Software
6) Go back to the flash drive where the "Setup" icon was found. Right click on "spreadsheet" and select 'Copy'
7) Go to the folder "c:\Programs Files\Turner Designs\Trilogy" (if c:\.....\Turner Designs\..... is not found in Program Files, select Program Files (x86) instead)
8) Right click and select "Paste"
9) Click "Yes" to Confirm File Replace
10) Go to Desktop and double click on the "Trilogy" icon to open the application program
11) Click "Select", then select COM Port # and click "OK"
12) Click on "Start"
13) A Berylliant spreadsheet opens
14) You are ready to calibrate and run samples. When finished save the spreadsheet with a different name in the folder of your choice.

(2) Optical Kit Installation/Removal

1. Turn Off the Fluorometer.
2. Grasp the handle of the Optical Kit and align with the sample compartment.

   Press down to lock the kit in place.

Optical Kit (FM-B)

3. Close the lid and turn On the Fluorometer.
4. On the touch screen press UV which identifies the Optical Kit. **(Note: <u>do not</u> select Custom followed by Fluorescence, the correct selection is UV for the Optical Kit)**

Instructions for Use of the Trilogy Software, the Trilogy Fluorometer and the Berylliant Spreadsheet

- Open the top of the fluorometer and install the custom optics component, pressing it firmly in place until a click is heard.
- Turn on the fluorometer and the computer.
- Click on the "Trilogy" icon located on your desktop.
- **The first time you use the software you must select the correct com port you are connected to on the computer. For future runs the correct com port will already be chosen for you.**
- Once the correct com port has been selected, click "Start" and the Berylliant spreadsheet will automatically open.
- **On the fluorometer touch screen, choose "UV" as the module being used.**
- Choose "Ok" to confirm that the new module is the "Fluorometer UV."

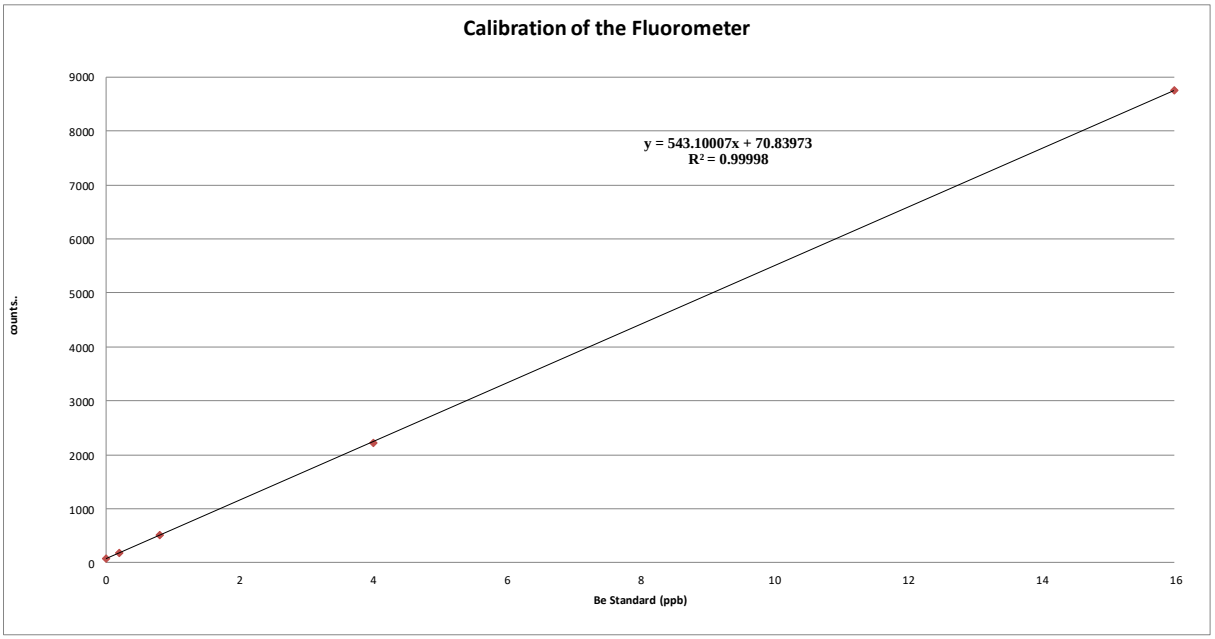
- To run a new calibration, choose “Calibrate.”
- Choose “Run New Calibration.”
- Choose the correct unit of measure, such as ppb.
- Follow the onscreen instructions to run the calibration standards starting with the blank. The cuvette should be placed into the sample compartment with the fluorometer lid closed when taking a reading. One blank and up to five standards can be used per calibration curve.
- When you are finished running all your standards, choose “Proceed with Current Calibration.”
- The next screen will say “Calibration completed. Would you like to save your calibration?” Touch “yes.”
- Enter a name for the calibration curve using the onscreen keyboard that pops up and save it. The Trilogy can store up to 18 calibration curves at one time. **(Note: if 18 calibration curves are stored new ones will not be saved. To save new calibration curves all or some of the stored curves must be deleted).**
- Choose “Calibrate.”
- Choose “Used Stored Calibration.”
- Touch the curve you want to use.
- Choose “View Details.”
- A calibration curve will appear on your Berylliant spreadsheet.
- Touch “Select” once or touch “Esc” twice to exit the calibration menu.
- You can proceed with reading your samples using either Direct Concentration Mode or Raw Fluorescence Mode.
    - To use the **Direct Concentration Mode** simply start reading your samples on the fluorometer, but **PLEASE NOTE** that your samples will **NOT** be read against the calibration curve you generated. They will instead be read against an internal fluorometer calibration based solely on the two

calibration standards your sample falls between. The readings will be in the unit of measure you calibrated with, such as ppb.

- To use the **Raw Fluorescence Mode** touch "Mode" before beginning reading samples. This will instantly change the mode to Raw Fluorescence Mode. Your readings will be in Fluorescent Standard Units (FSU). These readings will then need to be converted to the unit of measure you calibrated with, such as ppb. This can be done by substituting the FSU reading for "y" in the "y=mx+b" equation generated by your calibration curve, as seen on your graph on the Berylliant spreadsheet. You can then solve for "x" to give you the sample readings in the correct unit of measure, such as ppb.

- Before running a sample, you can label it on the Berylliant spreadsheet by choosing "Sample ID" in the upper left-hand corner of the screen. This will bring up a touchscreen keyboard where you can type in a name.
- Place your cuvette into the sample compartment, close the lid and choose "Measure Fluorescence" in the top right-hand corner of the screen.
- Once the measurement is done, the name and fluorescence data for your sample will automatically pop up in the same Berylliant spreadsheet as your standards.
- Continue doing this for the remainder of your samples.
- Be sure to save your data on the computer using a unique name, as sample data will not be saved on the fluorometer and the home screen keeps only 20 measurements onscreen before overwriting them.

Figure 3: Calibration Curve for 0.0, 0.5, 2.0, 10.0 and 40 ppb standards



The coefficient of determination (R^2) should be 0.999 or greater. If it is different from this, repeat the calibration process. If this does not resolve the problem, then from the Excel data you can check which of the standards is resulting in the highest error. That standard should be re-run or replaced with a new one and the calibration repeated.

1. If the fluorometer is not connected to a PC, then when the calibration is complete re-run the standards against the calibration. Values for each standard should read within 10% of the certified value, if not repeat the calibration and retest against the standard solutions. If this does not resolve the problem, prepare new "Standard Solutions" and repeat the calibration process. If still different, check the standards. Table 3 lists the values obtained on the fluorometer for the standards 0.0, 0.5, 2.0, 10.0 and 40.0 ppb, which were also used to calibrate the fluorometer. Results should be within 10% of the expected values.

Table 3: Check of calibration of the Fluorometer using standards as measured samples.

Standard Value (ppb)	Standard Read Against Calibration Curve (ppb)	% Difference
0.00	0.0	0.0%
0.50	0.49	-2.0%
2.00	1.99	-0.5%
10.00	10.0	0.0%
40.00	40.05	+0.13%

The standard calibration solutions in cuvettes must be stored in the provided amber jar when not in use. This will prolong their life and they may be used over two days. **It is recommended that the instrument be recalibrated after each time it is shut off or at least two times each day. More frequent calibration may be required if it is suspected that the temperature of the environment has changed by more than 3°C.**

2. To convert the data from ppb readings on the instrument to μg/ wipe, divide the ppb reading by 10. For example, a reading of 10 ppb on the instrument corresponds to 1 μg/100 cm^2, assuming that a 100-cm^2 area was wiped. **It is strongly recommended to only wipe 100 cm^2 area per sample, however, in the event the wiped areas are less than or greater than 100 cm^2, the results must be normalized to 100cm^2. For air samples the μg reading is normalized equivalent to 1,000 liter (or 1m^3) of air passed through the filter. For this normalization, the amount of air passed through the filter must be known.**

Appendix 3: Consumables kit (CO-A)

CO-1. Filter paper for wipes, 100 count

CO-2. Stencil, one count

CO-3. 15ml tubes with blue caps for dissolution of Be on wipe, 100 count

CO-4. Plastic syringes for filtration process of dissolution solution, 100 count

CO-5. Filters for syringes, 100 count

CO-6. 15 ml tubes with orange caps for storing analyte (filtered dissolution solution), 100 count

CO-7. Pipette tip to place 1.9 ml of detection solution in cuvet, 96 count

CO-8. Pipette tip to place 0.1ml of dissolution solution in cuvette, 96 count

CO-9. Cuvette (with cap) for fluorescence measurement, 100 count

CO-10. Pipette tip to place 5ml of dissolution solution in dissolution tube 100 count

Appendix 4: Beryllium Calibration Standards (ST-A)

Five bottles with 0 (S-B0), 10 (S-B10), 40 (S-B40), 200 (S-B200) and 800 ppb (S-B800) beryllium solution in ammonium bifluoride, 100 ml each. See Appendix 1

Consumables kit for Calibration Soln. Prep. (CC-A)

CC-1 Amber bottle to temporarily store up to 6 cuvettes with standards, one count

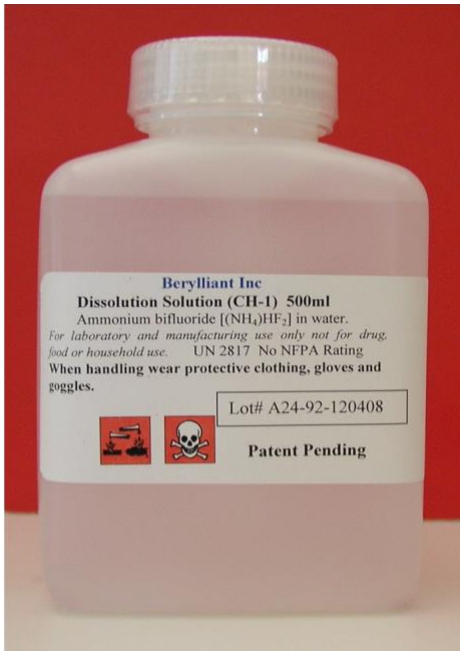
CC-2. Pipette tip to dispense 1.9 ml of detection solution in cuvettefor standards, 96 count

CC-3. Pipette tip to dispense 0.1 ml of standard solution in a cuvette, 480 count

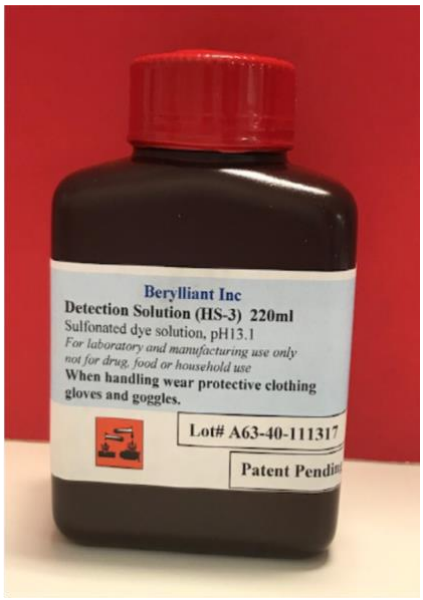
CC-4. Cuvette(with cap) for fluorescence measurement from standards, 500 count

Appendix 5: Chemicals Kit (CH-A)

Included in the terms of purchase of this product is a limited license from Berylliant Inc for its use to detect beryllium using the technology owned by Berylliant. Further, the technology rights from Berylliant Inc under this license are only conveyed when equipment and materials are purchased from Berylliant Inc or its appointed agent. The technology from Berylliant is covered under several patents and pending patents, a list of which can be obtained from Berylliant Inc.



CH-1. Dissolution solution, 500 ml

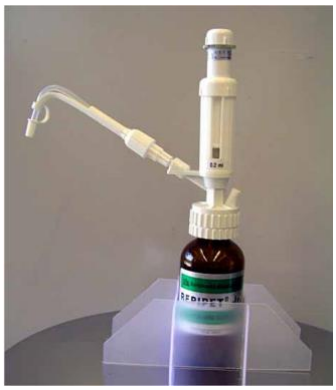


HS-3. Detection solution, 220 ml

CH-1 is available in 250ml, 500ml and 1000ml and HS-3 in 110, 220 and 1000ml quantities.

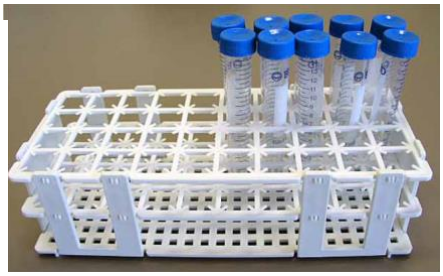
.

Appendix 6: Sample Processing Kit (PR-A)



PR-1. Water dispenser to wet wipe

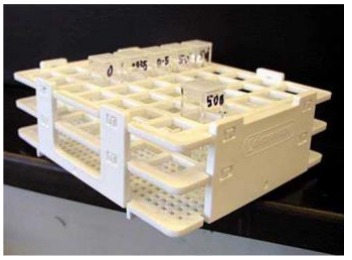
PR-2. Pipetter to dispense 5 ml of dissolution solution



PR-3. Stand to hold 40 tubes of dissolution solution w/wipes

PR-4b. Heating block for dissolution tubes, or see Fig 1 for PR-4 (rotator)

PR-5: Stand to hold 40 tubes of filtered dissolution solution.

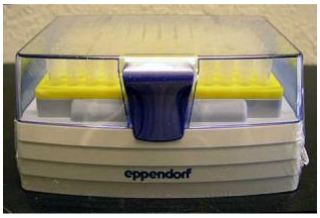


PR-6. Stand to hold 42 cuvettes

PR-7. 2.5 ml Pipetter to dispense 1.9 ml of detection (dye) solution into cuvettes (for samples and for standards)

PR-8. 0.1ml Pipetter to dispense 0.1 ml of dissolution solution into cuvettes (for samples and for standards)

PR-9. Stand for pipetters



PR-10: Box for 0.2ml pipette tips

PR-11: Box for 2.5ml pipette tips

PR-12: Box for 5ml pipette tips

Appendix 7: High Resolution Measurements

Quantification of beryllium at or below 0.001µg (1ng) of beryllium in media Applicable to wipes and air filters

Among other things, the quantification level from this method depends on the fluorometer used. A high-resolution process is described below, using the same equipment and materials. This method extends the quantification limit by a factor of 10, i.e., from 0.05 µg on the media, down to starting from 0.005µg on the media. An ultra-high resolution can also be obtained where beryllium can be quantified at 0.001µg (or 1ng) on the media. The samples which show beryllium levels of below 0.05 µg or are non-detect using standard procedure (20X dilution), may be re-analyzed using these procedure modifications. These procedures mainly require preparation of a different set of calibration solutions and analysis solutions using a different dilution ratio as opposed to 20X in the standard procedure. All precautions, dissolution and filtration processes, setting of optical filters, calibration regression coefficients and thermal control are the same, as explained in the previous sections (see reference 7 in General References section for ultra-sensitive range measurement). The high resolution step uses a dilution ratio of 5X and the ultra-high resolution method uses a dilution ratio of 3X. Either may be used if the samples are found to be non-detect using the standard procedure (use of 20X dilution) depending on the requirements. **Please note the 3X procedure only works when using the dye solution HS-3.**

High Resolution Measurement (5X dilution)

Preparation of calibration solutions

1. Using the standard calibration solutions comprising 0, 10, 40, 200 and 800 ppb, a new set of diluted standards (10X dilution) is made using the pipettes and the pipette tips supplied by taking 0.2ml of these standards and adding 1.8ml of ammonium bifluoride dissolution solution, as shown in column 2 of Table 4. The shelf life of 1 and 4ppb standard solutions can easily change with time, thus this

should to be done every time this procedure is used. The standards may be made in cuvettes or centrifuge tubes. Alternatively, one may purchase a 10X dilute set of standards from Berylliant Inc.

2. Using these standards, measurement solutions for calibration are made, as shown in column 3 of Table 4. Diluted standard in a quantity of 0.4ml is added to 1.6ml of the detector solution (this step is called 5X dilution, as the measurement solution has $1/5^{th}$ of the dissolution solution comprising beryllium).
3. These solutions are measured, and a calibration curve is established. These levels of beryllium correspond to the amount of beryllium on the media, as given in column 5 of Table 4.

Table 4: 5X Dilution High Resolution Measurements

Amount of beryllium in standard, ppb	Amount of Be in diluted standard (1.8ml of dissolution solution+0.2ml of standard), ppb*	Amount of Be in measurement solution (1.6ml of detector solution and 0.4ml of diluted standard), ppb	Amount of beryllium in µg /ml of measurement solution	Amount of Be (µg) in a wipe after wiping 100 sq cm
0	0	0	0	0
10	1	0.2	0.0002	0.005
40	4	0.8	0.0008	0.02
200	20	4	0.004	0.1
800	80	16	0.016	0.4

** Rather than make 10x dilution standards, the user may purchase 10X dilute standards from Berylliant Inc*

Preparation of samples for measurement and their measurement

1. 0.4 ml of filtered dissolution solution (which may comprise beryllium) is added to a cuvette along with 1.6ml of the dye solution. **Please do not use** the sample solutions from the standard procedure (20X dilution), which have 0.1ml of the dissolution solution comprising beryllium and 1.9ml of the dye solution, (see Table 2 for comparison).
2. After establishing the calibration curve as explained above, the solutions can be measured on the fluorometer in the usual manner.

Ultra High Resolution Measurement (3X dilution)

Preparation of calibration solutions

1. Using the standard calibration solutions comprising 0, 10, 40, 200 and 800 ppb, a new set of diluted standards (100X dilution) is made using the pipettes and the pipette tips supplied. It is suggested that this is done in two steps where each step is a 10X dilution.
    a. The first 10X dilution is done by by taking 0.2ml of these standards and adding 1.8ml of ammonium bifluoride dissolution solution, as shown in column 2 of Table 4. The shelf life of 1 and 4ppb standard solutions can easily change with time, thus this should to be done every time this procedure is used. The standards may be made in cuvettes or centrifuge tubes. Alternatively, one may purchase 10X dilute set of standards from Berylliant Inc.
    b. The second 10X dilution is done by by taking 0.2ml of the already diluted standards in step "a" and adding 1.8ml of ammonium bifluoride dissolution solution. The shelf life of these standard solutions can easily change with time, thus this should be done every time this procedure is used. The standards may be made in cuvettes or centrifuge tubes. **One should absolutely ensure that the pipettes are well calibrated, as any error in making standards will permeate through all the results**.
2. Using these standards, measurement solutions for calibration are made as shown in column 3 of Table 5. Diluted standard in a quantity of 0.67ml is added to 1.33ml of the detector solution (this step is called 3X dilution, as the measurement solution has 1/3rd of the dissolution solution comprising beryllium).
3. These solutions are measured, and a calibration curve is established. These levels of beryllium correspond to the amount of beryllium on the media, as given in column 5 of Table 4.

Table 5: 3X Dilution High Resolution Measurements

Amount of beryllium in standard, ppb	Amount of Be in **twice** diluted standard (1.8ml of dissolution solution+0.2ml of standard), ppb*	Amount of Be in measurement solution (1.33ml of HS-3 solution and 0.67ml of diluted standard), ppb	Amount of beryllium in µg /ml of measurement solution	Amount of Be (µg) in a wipe after wiping 100 sq cm
0	0	0	0	0
10	0.1	0.033	0.000033	0.0005
40	0.4	0.133	0.000133	0.002
200	2	0.667	0.000667	0.01
800	8	2.67	0.00267	0.04

Preparation of samples for measurement and their measurement

1. 0.67 ml of filtered dissolution solution (which may comprise beryllium) is added to a cuvette along with 1.33ml of the HS-3 dye solution.
2. After establishing the calibration curve as explained above, the solutions can be measured on the fluorometer in the usual manner.

Three Months Limited Warranty

Berylliant, Inc. ("BERYLLIANT") warrants the products supplied by BERYLLIANT to the limited extent provided herein against defects in materials and workmanship. The warranty period for all products supplied by BERYLLIANT is for three (3) months from the first to occur of (i) the date the product is sold by BERYLLIANT or (ii) the date the product is purchased by the original retail customer (the "Commencement Date"). Except as expressly stated above, BERYLLIANT MAKES NO OTHER WARRANTY EXPRESSED OR IMPLIED, WITH RESPECT TO THE PRODUCTS AND EXPRESSLY DISCLAIMS ANY AND ALL WARRANTIES, INCLUDING BUT NOT LIMITED TO, WARRANTIES OF DESIGN, MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE.

An authorized representative of BERYLLIANT must perform all warranty inspections. In the event of a defect covered by BERYLLIANT's warranty, BERYLLIANT shall, as its sole obligation and exclusive remedy, provide free replacement parts to remedy the defective product. In addition, for product kits PR-A sold by BERYLLIANT within the continental United States or Canada, BERYLLIANT shall provide free labor to repair the products with the replacement parts, but only for a period of ninety (90) days from the Commencement Date.

BERYLLIANT's warranty provided hereunder shall be null and void and without further force or effect if there is any (i) repair made to the product by a party other than BERYLLIANT or its duly authorized service representative, (ii) misuse (including use inconsistent with written operating instructions for the product), mishandling, contamination, overheating, modification or alteration of the product by any customer or third party or (iii) use of replacement parts that are obtained from a party who is not an authorized dealer of BERYLLIANT.

As a condition to the return of any product, or any constituent part thereof, to BERYLLIANT, it shall be sent prepaid and a prior written authorization from BERYLLIANT assigning a Return Goods Number to the product or part shall be obtained.

IN NO EVENT SHALL BERYLLIANT BE LIABLE TO ANY PARTY FOR ANY DIRECT, INDIRECT, SPECIAL, INCIDENTAL, OR CONSEQUENTIAL DAMAGES, OR FOR ANY DAMAGES RESULTING FROM LOSS OF USE OR PROFITS, WHETHER ANTICIPATED OR OTHERWISE, ARISING OUT OF OR IN CONNECTION WITH THE SALE, USE OR PERFORMANCE OF ANY PRODUCTS, WHETHER SUCH CLAIM IS BASED ON CONTRACT, TORT (INCLUDING NEGLIGENCE), ANY THEORY OF STRICT LIABILITY OR REGULATORY ACTION.

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