



Manual for Procedures and Kit Description

For

Determination of Beryllium Particulates (BeFinder®)*

Portable System

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 the fluorescence detection hardware is 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.

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

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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)

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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 when combined with a dye by fluorescence. The remaining solution may be used for future reference or used 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 (CH-2)	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	Promega GloMax [®] - Multi Jr. Fluorometer and optical Kit FM-B	Appendix 2
Calibration Standards	ST-A 3rd Party order (Appendix 1)	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 repeatedly for several 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 Promega GloMax[®]-Multi Jr. 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 of 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 25**. 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 22**.

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 into heating unit. Set the temperature to 85°C for dissolution of the beryllium on the media.
5. Open box for the pipetter stand (PR-9) and place it on the table.
6. Open box for 5ml pipette (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 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 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 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 pipettors should be periodically checked for accuracy and adjusted if required, e.g., a recommended period is every 3 months.

A Promega GloMax[®]-Multi Jr. fluorometer (FI-B) is provided by Berylliant Inc. This is supplied with an optical kit (FM-B) with an excitation filter at $380\text{nm} \pm 15\text{nm}$ and emission filter at $480\text{nm} \pm 5\text{nm}$. 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).

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 0.005 μg or above in the media. Limit of detection for the method has been established at 0.0008 μg .

The procedure described here uses a Promega GloMax[®]-Multi Jr. fluorometer, Model E6070. 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-9 on **page 22, Appendix 3**)
- Calibration standards (ST-A on **page 23 Appendix 4**)
- Calibration associated consumables (CC-1 to CC-4 on **page 23, Appendix 4**)
- Chemicals (CH-1 and CH-2 on **page 24, Appendix 5**) and
- Sample processing items (PR-1 to PR-11 on **page 25, Appendix 6**).

1. Wipe Collection

- a. Wet filter paper (CO-1) with 0.2 ml DI water using dispenser (PR-1), and wipe a 100cm² 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) and push it inside, cap and label tube.
- c. Collect as many samples as desired, each in a separate dissolution tube.

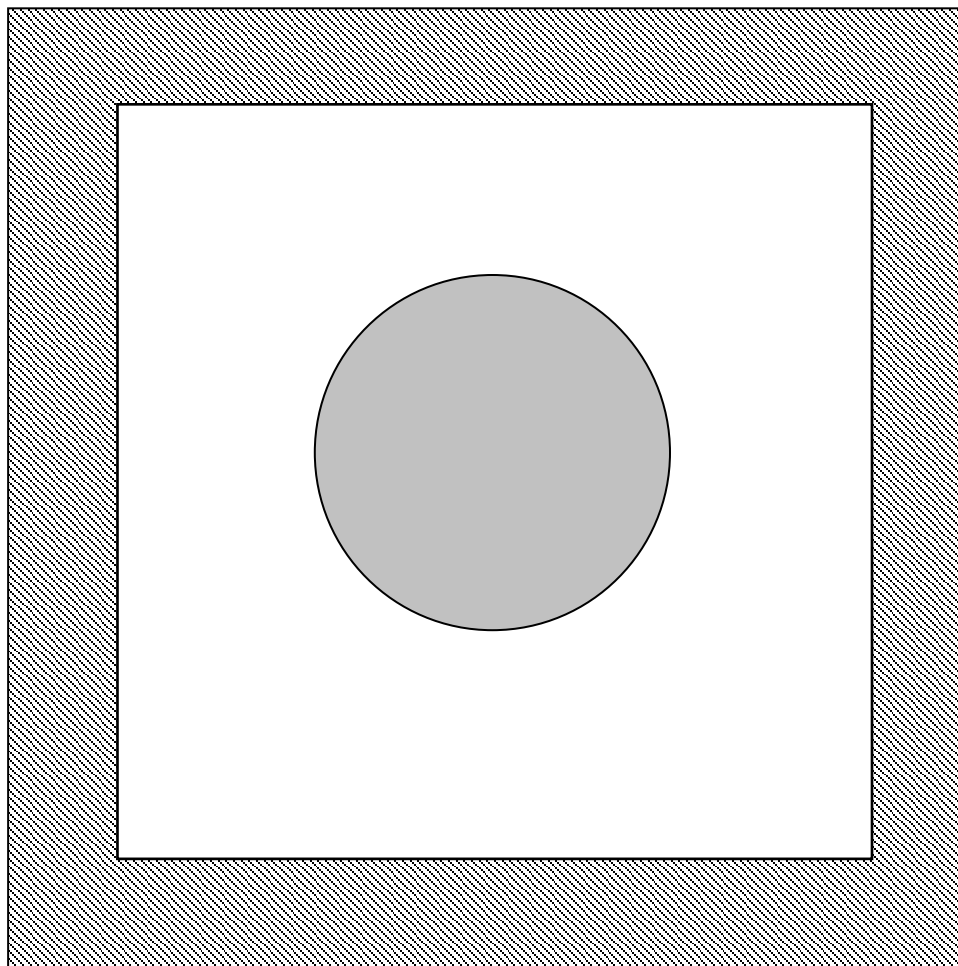


Figure 1: This shows the square stencil (CO-2) to visualize the 10 cm x10 cm wipe area. The round filter paper (CO-1, 47mm diameter) to wipe the surface is also shown to demonstrate its relative size to the 10 cm x10 cm area.

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 stand (PR-3).
- d. 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.
- 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 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 dissolved sample) into a labeled cuvette (CO-9). Cuvettes suitable for fluorescence work should be used. The cuvettes may be put in cuvette stand (PR-6). 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 (CH-2) into an analyte solution in a cuvet. Aspirate/de-aspirate solution several times to mix properly. Cap the cuvette. Repeat this process for all samples using separate cuvettes. The solution must be clear and colorless. A yellow-gold color shows presence of iron. The solution 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 PALL 0.2 μ m GHP ACRODISC Luer-lok™ filter into a new cuvette. Mixing of 0.1ml of dissolution solution to 1.9 ml of the dye solution is called 20X dilution. This allows beryllium to be quantified from 0.05 μ g on the media. On the other hand use of 5X dilution (see Appendix 7) allows quantification from 0.005 μ g on the media.

4. **Calibrate the instrument** (See Appendix 1 (*Procedure to prepare calibration standards*) and Appendix 2 (*Instructions for Quantitative Analysis of Beryllium Using the Promega GloMax®-Multi Jr. Fluorometer and Calibration*). See Appendix 7 if beryllium quantification is desired from 0.005 μ g on the media.

5. Measurement of collected samples

- a. After the instrument is calibrated using the prepared standards i.e. 0, 0.5, 2.0, 10.0 and 40.0 ppb.

- b. Insert cuvette with collected (unknown) sample in the sample compartment, close 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 $\mu\text{g}/\text{wipe}$, divide the ppb reading by 10. For example, a reading of 10 ppb on the instrument corresponds to 1 $\mu\text{g}/100\text{ cm}^2$, 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 (1 m^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 repeatedly over several days.

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 is 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 100cm² 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 or the spectrometer using 0, 10, 40, 200 and 800 ppb standards prepared by adding 0.1 ml of standards to 1.9 ml of the detection solution (20X dilution). These prepared calibration solutions may be re-used up to a few days as long as they are stored in capped bottles or cuvettes and away from light below 450 nm wavelengths and 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, McCleskey 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) (Please see below)
2. Berylliant detection solution (**CH-2, page 24**)
3. 2 ml cuvettes (ensure that cuvettes for fluorescence work are used) with caps (**CC-4, page 23**)
4. Dispensing pipettors 0.1 ml fixed volume (**PR-8, page 25**) and 0.5 to 2.5 ml variable volume (**PR-7 page 25**) and corresponding tips **CC-3 and CC-2 (page 23)**.

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

Procedure

1. Take 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 (CH-2). Take 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 cuvet. Aspirate/de-aspirate solution several times and/or stir with the pipette tip to mix properly. 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 23**)

These are all Custom Claritas Standards (PPT Grade) with six-month expiration
Matrix: 1% Ammonium bifluoride/H₂O/trace HNO₃

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 **Promega GloMax[®]-Multi Jr Fluorometer** (2)
Measuring Beryllium Standards and Storing Calibration Curves.

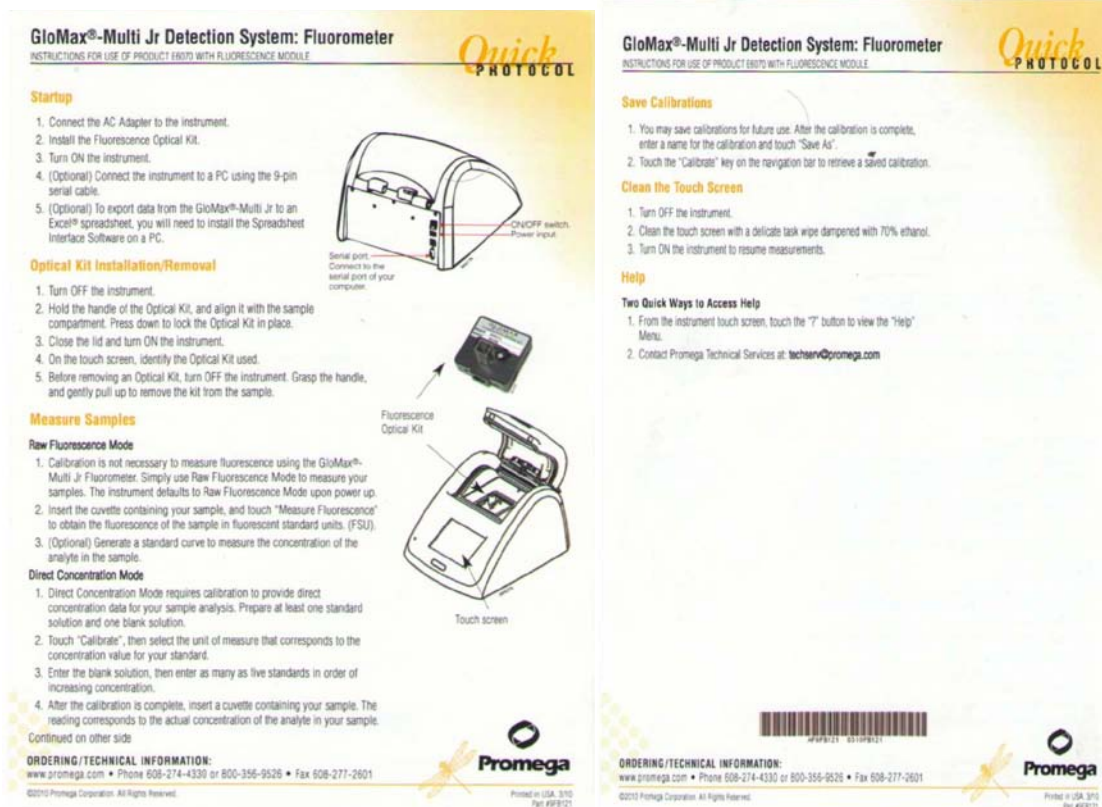
Figure 2: Promega GloMax[®]-Multi Jr. Fluorometer



FI-B

The Fluorometer is UL listed

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



(1) Connections

1. Connect the power supply to the Promega GloMax®-Multi Jr. 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 s Fluorometer to a PC using the 9 pin serial cable (optional).
5. Install the Spreadsheet Interface Software on a PC to import data from the Fluorometer to an Excel spreadsheet as follows:

Installation Instructions

This procedure will install the Glomax software and replace the original spreadsheet with the Berylliant spreadsheet.

Connect the unit to the RS232 serial port on your computer (if your computer does not have a RS232 serial port use the USB/RS232 SERIAL adapter)

Glomax CD and Berylliant spreadsheet Installation Instructions for Windows XP users:

- 1) Insert the CD and follow the screen instructions to install the Glomax Spreadsheet Interface Software
- 2) After the Program is installed successfully, Go to “My Computer” select the CD [drive] and right click on it and select “explore”
- 3) Once on the “CD[drive]” folder right click on spreadsheet and select ‘Copy’
- 4) Go to the folder “c:\Program Files\Promega\Spreadsheet Interface Software\”
- 5) Right click on it and select “Paste”
- 6) Click “Yes” on Confirm File Replace
- 7) Go to Desktop and double click on “Spreadsheet Interface Software” Icon to open the application program
- 8) Click “Select”, then select COM Port # and click “OK”
- 9) Click on “Start” and select “Read Only”
- 10) A Berylliant spreadsheet opens.
- 11) You are ready to calibrate and run samples. When finished save the spreadsheet with a different name in the folder of your choice.

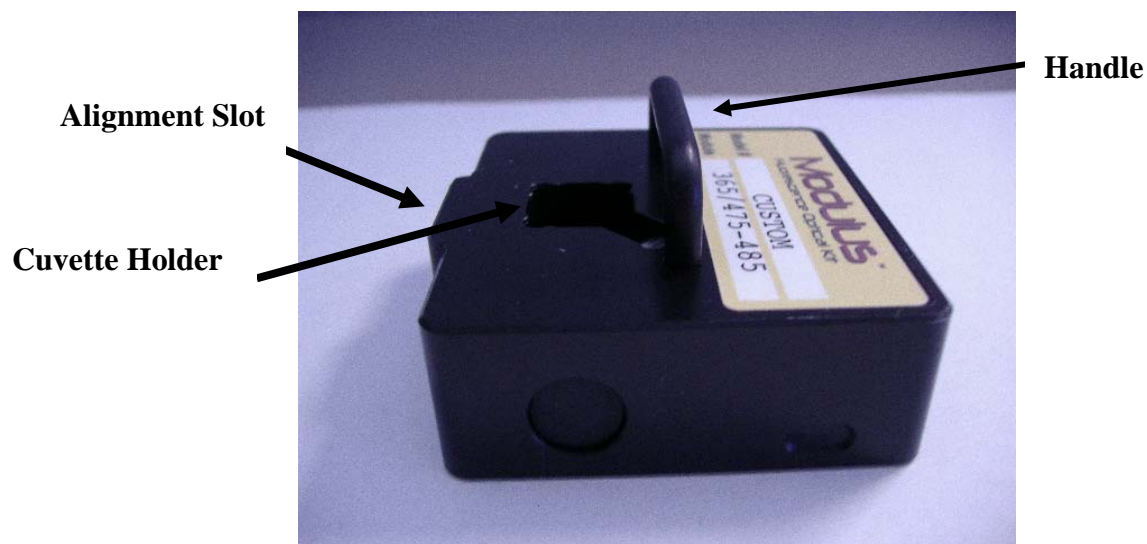
Glomax CD and Berylliant spreadsheet Installation Instructions for Windows Vista/ 7 users:

- 1) Insert the CD, run “setup.exe” and follow the screen instructions to install the Glomax Spreadsheet Interface Software
- 2) After the Program is installed successfully, Go to Computer and select the CD drive. Right click on the CD[drive] and select “Open”
- 3) Once on the “CD[drive]” folder ,select “spreadsheet” and select ‘Copy’
- 4) Go to “Computer”, then folder “c:\Program Files\Promega\Spreadsheet Interface Software\” (if c:\.....\Promega\... is not found in Program Files select Program Files(x86) instead,)
- 5) Right click on ... \Spreadsheet Interface Software\ and select “Paste”
- 6) Click on “Copy and Replace” then click on “Continue”
- 7) Go to Desktop and click on “Spreadsheet Interface Software” Icon to open the application program
- 8) Click select COM Port #, then click “OK”
- 9) Click on “Start” and select “Read Only”
- 10) A Berylliant spreadsheet opens.
- 11) 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: do not select Custom followed by Fluorescence, the correct selection is UV for the Optical Kit**)

Instructions for Setup of the Promega GloMax[®]-Multi Jr. Fluorometer Software

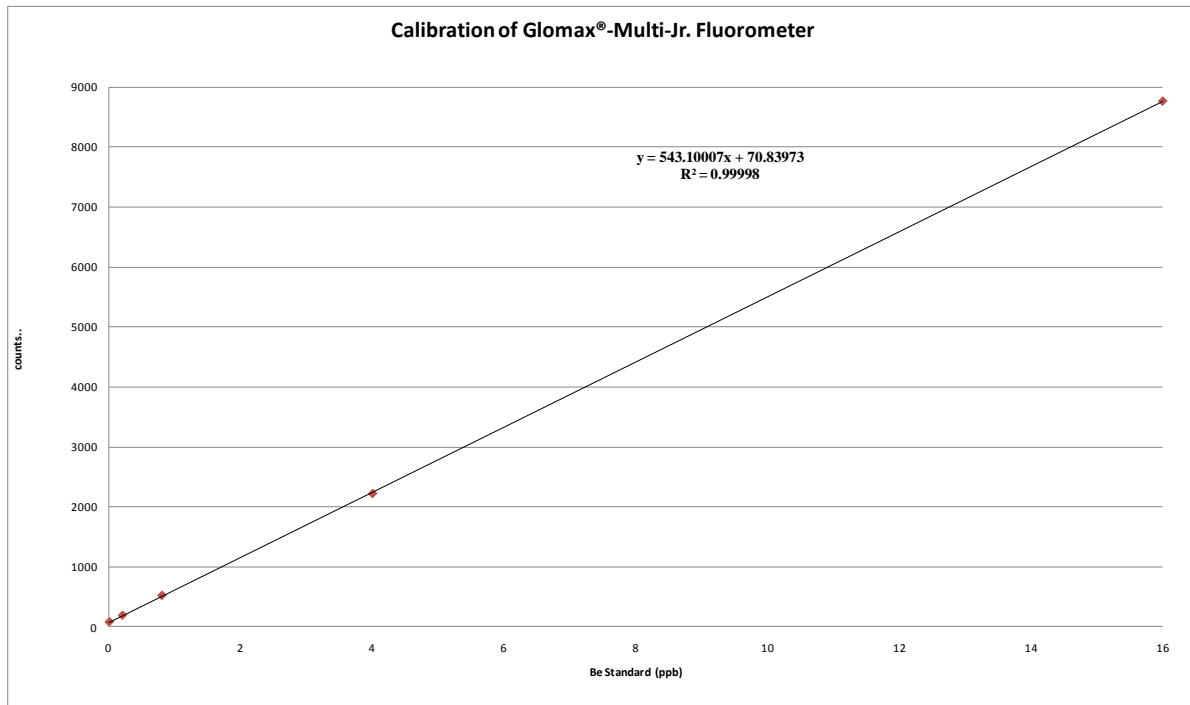
- Open the top of the fluorometer and install the custom optics component pressing it firmly in place until a click is heard.
- Turn on the fluorometer.
- To setup the Glomax software, first connect the RS-232 serial cable to the Glomax and then to the computer.
- Insert the Glomax Software CD into the CD drive of your computer.

- After a few seconds the “Spreadsheet Interface Software Setup Wizard” screen will appear.
- Click “Next.”
- Click “Next” again.
- Click “Next” to install the folder.
- Click “Install.”
- Click “Finish.”
- An icon called “Spreadsheet Interface Software” will appear on your desktop.

Instructions for Use of the GloMax Software and the GloMax[®]-Multi Jr. Fluorometer

- 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 “Spreadsheet Interface Software” icon located on your desktop.
- **The first time you use the software you have to 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 a box will pop up asking for a password for write access. Click “read only” and an Excel sheet will automatically pop up.
- 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.
- 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 of your standards, choose “Proceed with Current Calibration.”
- The next screen will say “Calibration completed. Would you like to save your calibration?”
- Enter a name for the calibration curve using the onscreen keyboard that pops up and save it. The Glomax 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).**
- Touch the house icon.
- Choose “Calibration.”
- Choose “Used Stored Calibration.”
- Choose “View Details.”
- The concentration and raw fluorescence data for the blank and the standards will appear on the computer in the Excel sheet and will show a calibration curve, as seen in Figure 3 below.
- Touch the house icon. This will take you back to the starting screen.
- To run samples, choose “Sample ID” in the upper left hand corner of the screen. This will bring up a touch screen keyboard where you can name your sample by typing a name in.
- Place your cuvette into the sample compartment, close the lid and choose “Measure Fluorescence.”
- Once the measurement is done, the name and fluorescence data for your sample will automatically pop up in the same Excel sheet as your standards.
- Continue doing this for the remainder of your samples.

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 obtain on the Promega GloMax[®]-Multi Jr. 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 5% of the expected values.

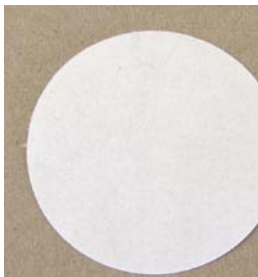
Table 3: Check of calibration of the Promega GloMax® -Multi Jr. Fluorometer using standards as measured samples.

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 repeatedly over several 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 $\mu\text{g}/\text{wipe}$, divide the ppb reading by 10. For example, a reading of 10 ppb on the instrument corresponds to 1 $\mu\text{g}/100\text{ cm}^2$, assuming that 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 100 cm^2 .** For air samples the reading μg is normalized equivalent to 1,000 liter (or 1 m^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,



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



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



CO-5. Filters for syringes, 100



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



CO-7. Pipette tip to place 1.9 ml of detection solution in



CO-8. Pipette tip to place 0.1ml of dissolution solution in



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



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

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 cuvette for standards, 96 count



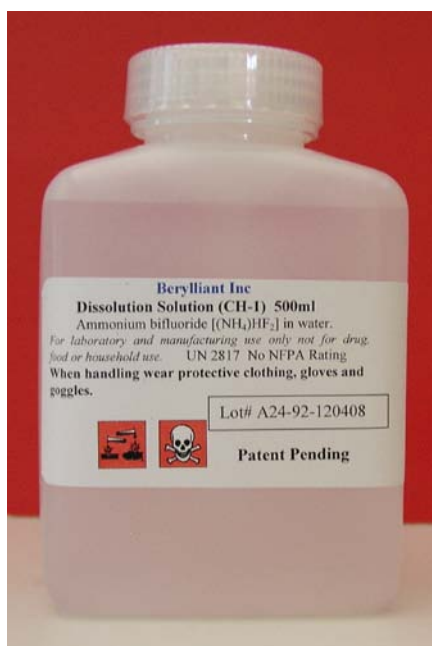
CC-3. Pipette tip to dispense 0.1 ml of standard solution in a cuvette, 480 count



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 the fluorescence detection hardware is 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 is available in 250ml and 500ml and CH-2 in 110, 220 and 1000ml quantities.

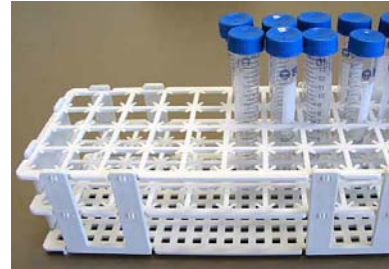
Appendix 6: Sample Processing Kit (PR-A)



PR-1. Water dispenser to wet



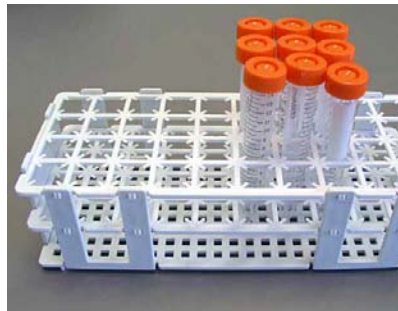
PR-2. Pipetter to dispense 5 ml of dissolution solution



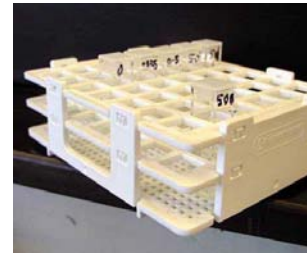
PR-3. Stand to hold 40 tubes of dissolution solution



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



PR-6. Stand to hold 42 cuvettes



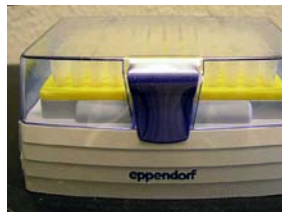
PR-7. 2.5 ml Pipetter to dispense 1.9 ml of detection (dye) solution into cuvettes (for samples and for



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



PR-9. Stand for pipettors



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 0.02µg 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 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 this procedure modification. This procedure mainly requires preparation of a different set of calibration solutions and analysis solutions using a dilution of 5X 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).

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 10X dilute set of standards (from the same vendor as listed in Appendix 1).
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 1/5th 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 $\mu\text{g}/\text{ml}$ 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 Spex Certiprep, see Appendix 1)

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.

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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 products 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.

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***Users are responsible for choosing and using BeFinder system at their own risk. Berylliant is not responsible for any damages caused by the use of the system.**