

Standard Operating Procedure

SOP109

Title: Use and Maintenance of the BMG FLUOstar Omega Plate Reader

Location: CBE Laboratory Unit (H21)

1. PURPOSE

To describe the procedures for the use and maintenance of the FLUOstar Omega micro plate reader

2. SCOPE

This SOP applies to operational and maintenance procedures for the FLUOstar Omega micro plate reader, located in the CBE Laboratory Unit at Holywell Park. The multifunctional plate reader supports a wide variety of applications and the following reading modes; fluorescence intensity, time-resolved fluorescence, and absorbance (luminescence is a potential optional extra). The micro plate reader is designed to detect biological, chemical, or physical events of samples. It can be used for a variety of assay procedures for example, the detection of cell toxicity, proliferation and viability, enzyme activity, protein expression and cell growth.

Important Restrictions: This SOP does not apply to the use and operation of the plate reader under atmospheric control conditions. The use of the atmospheric control unit (ACU), enabling additional control of O₂/CO₂ within the incubator chamber, requires connection of CO₂ and N₂ gas supply, which when connected for the first time must be subject to the completion of a risk assessment and revision of this SOP.

3. RESPONSIBILITIES

- (i) All personnel who are authorised to operate the FLUOstar Omega plate reader shall ensure the proper operation of the instrument as outlined in this document and the Operating Manual.
- (ii) The Laboratory Manager (LM) or Responsible Person (RP) shall schedule service and preventative maintenance with authorized service representatives.
- (iii) The LM/RP shall ensure that prior to authorising the commencement of maintenance, repairs or annual certification testing that the FLUOstar Omega plate reader is suitably disinfected in accordance with SOP003 and that a "Decontamination Certificate" is issued.
- (iv) Authorized users are expected to have received appropriate and documented training. Before a new user is allowed to operate the FLUOstar Omega plate reader on their own, training and competence to operate the equipment must have been assessed by the responsible person or another experienced user.
- (v) The LM/RP shall provide the following for users of the FLUOstar Omega plate reader:
 - information on the hazards and risks to health.
 - instruction in safe procedures;
 - training, where necessary; and effective supervision to ensure, so far as is reasonably practicable, that the plate reader is operated without risks to the health of employees and other persons, i.e. including students and visiting research workers.

Version 002

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Written by: E.Ratcliffe	Reviewed by: P.Hourd	Approved by: R.I.Temple
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4. EQUIPMENT AND MATERIALS

- (i) FLUOstar Omega microplate reader
- (ii) 70% IMS
- (iii) Multiwell (e.g. 96 well) assay plates

5. PROCEDURE

NOTE: Before using the plate reader, users should refer to the FLUOstar Omega Operator Manual and associated CBE risk assessment for further recommendations on safe operation.

5.1 General Controls and Precautions

- (i) Always wear appropriate PPE when working with the Micro plate reader e.g. lab coat, gloves (Refer to SOP037).
- (ii) Check that electrical test labels are current. If not do not use and inform the LM/RP.
- (iii) Use of the micro plate reader may involve handling specimens or reagents that are potentially hazardous. Always refer to the appropriate risk assessments.
- (iv) If moving the instrument to another location, ensure that transport lock is in the locked position (refer to operator manual for moving the machine).

5.2. Equipment Set-up Options

- (i) Refer to operator manual for description of components, and for instructions to install and change optics. The Omega readers are equipped with quick-fix mountings allowing for easy exchange of optics and placement of position spacers. No tools are required for changing the optics.

CAUTION: Always hold the optics by the black mounting piece.

- (ii) Filters; 4 excitation and 4 emission filters are factory installed. All BMG filters have an arrow printed on the side to indicate the direction in which they should be installed. The arrow should point in the same direction as the light. Refer to operator manual for more information on filter installation

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The filters installed are as follows;

Filters:	1	2	3	4	5	6	7	8
Excitation:	355	544	485BP12	584				Abs Ring
Emission:	460	590	Em520	620BP12				UV lens

NOTE: The FLUOstar Omega is also fitted with an Absorbance filter (Abs Ring) for PMT based Absorbance

CAUTION: If the instrument makes a grinding sound it is very likely that the large filter wheel nut in the middle should be tightened better or that a filter screw is loose.

- (iii) Spacers: the plate reader is designed for most microplate formats. The height of some microplates exceeds the space allowed under the optic. The minimum space between the optic and the microplate should be 1.5mm. With 6, 24, 48-well plate formats, it will be necessary to raise the optic using the spacers provided in the service box. The spacers are metal rectangular pieces with a hole in the centre. Each spacer is 2mm in height. They will be installed between the measurement head and the bottom of the reagent box (refer to operator manual for positioning). The number of spacers depends on how high the optic needs to be elevated. Determining the number of spacers and spacer installation is described with reference figures in the operator manual.
- (iv) **NOTE:** The FLUOstar Omega is not currently equipped with reagent injectors. The Omega readers can be equipped with up to 2 reagent injectors.
- (v) There are 2 parts to the Omega software; the Control part and the Data Analysis part. The control software is necessary for configuring the instrument, for setting up test parameters and for executing measurement procedures. The MARS data analysis software can be installed on office computers for off-line analysis. Refer to the Software manual for detailed instruction on use of the software.
- (vi) Quick Guide Omega Software – A copy is located with the plate reader and a copy is shown in Section 5.3.

5.3. Start up and Operation

- (i) Switch on the plate reader and associated computer and follow the instruction guide reproduced below:

NOTE: A constant green light (on front of machine) means the instrument is turned on. A flashing green light means the instrument is busy (e.g. performing a measurement, plate in/out, priming etc). A faster flashing green light (5 flashes per second) means an error has occurred (refer to operator manual).

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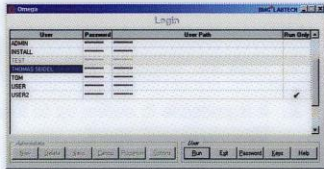
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Quick Guide Omega Software

STARTUP

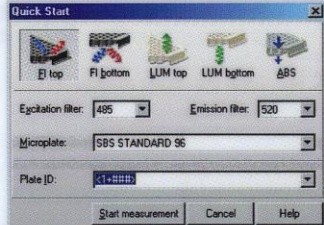
- Turn on the instrument and the computer.
- Start the **Omega Control** software.
- Login with your password or just click 'Run' to login as "User".



To measure a microplate, you can either use the quick start function or you can execute a pre-defined test protocol.

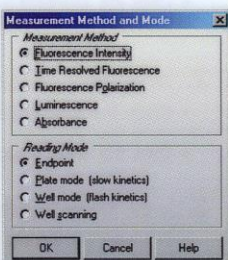
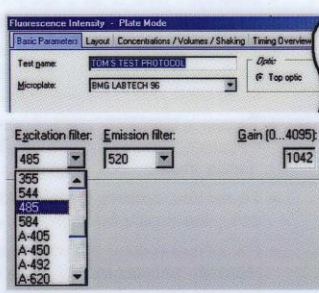
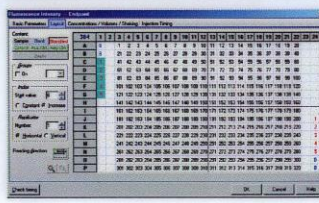
QUICK START

1. To measure a full plate in endpoint mode without defining a test protocol, click the 'Quick Start' button:
2. Select the measurement method. Choose the **excitation** and **emission filters** and the type of **microplate** that will be used.
3. A plate identifier (**Plate ID**) can also be specified (optional).
4. Start the measurement.



PROTOCOL DEFINITION

1. To create a new **test protocol** or to edit an existing one:
 - Click the 'Test protocols' button:
 - Double click the **protocol name** to edit an existing protocol or click 'New' to create a new protocol. Choose the *Measurement Method* (FI, FP, TRF, luminescence, absorbance) and choose the *Reading Mode*:
 - **End point** for single readings
 - **Plate mode** for slow kinetics
 - **Well mode** for fast kinetics
 - **Well scanning** for scanning (useful if you use large wells and if the samples are not equally distributed)
2. Inside the protocol definition window:
 - Enter a **test protocol name**.
 - Choose the **microplate** being used (Greiner, Corning, Nunc, etc.).
 - Type in a **positioning delay** (0.2s for non-cell based assays, or else 0.5 s).
 - **Plate Mode**: Type in the **no. of cycles** (how many times the reader will cycle through the plate).
 - **Well Mode**: Type in the **no. of intervals** (how many times the reader will read the well).
 - Type in the **no. of flashes** to be used per reading (default settings are recommended).
 - Choose the **excitation** and **emission filters** to be used.
 - Select the '**Layout**' sheet. Enter the position of samples, blanks and standards (if any).
 - If standards and/or reagent dispenser(s) are used, type in the values in the '**Concentrations / Volumes / Shaking**' window.
 - Click the '**Check timing**' button. This gives you the smallest possible cycle time (*Plate Mode*) or interval time (*Well Mode*). A longer time can be achieved by typing in a higher value in the '**Basic Parameters**' sheet.

0415F0005A Quick Guide Omega 1


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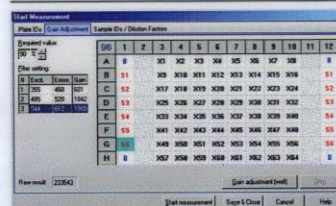
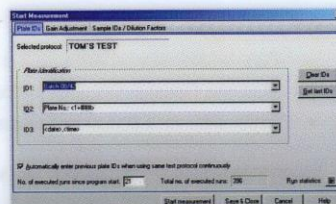
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Quick Guide Omega Software

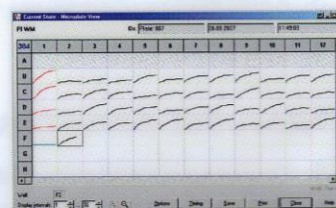
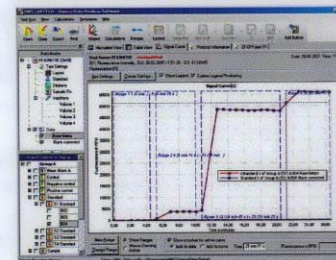
MEASURING (executing pre-defined protocols)

1. Click the 'Measure' button: 
2. It is possible to define up to three plate identifiers in the 'Start Measurement' sheet.
3. In the 'Gain Adjustment' sheet, select the well that will have the highest intensity and click the 'Gain adjustment' button:
 - The **required value** should be 90% in endpoint readings (giving highest values around 260 000 - 10% = 234 000).
 - For kinetic measurements, 10% - 50% could be the required value (this is dependent on the expected increase in the signal).
4. Click the 'Start measurement' button.



RESULTS

1. To see the measurement results during a reading:
 - Click the 'Current State Graphics' button . Different display options are available, e.g. curve, spectra...
2. To perform data calculations using the **Data Analysis** software:
 - Close the 'Current State' window.
 - Click the 'Data Analysis Software' button: 
3. In the 'Open Test Runs' window:
 - Double click the **test name** of the test run to be analyzed
4. Analyze the measured data:
 - Select the data to be displayed in the working area with the navigation tree (Data Node) on the left side of the main window.
 - Use the standard calculation wizard to perform a quick curve fit calculation; or use the calculation menus to define what is to be calculated and to be displayed.
 - To see a standard curve, open the 'Standard Curve' page. The calculated unknowns are displayed in the 'Microplate View' and the 'Table View'.
 - To remove outliers, simply shade them out in the 'Microplate View' using the toggle function (Ctrl -T).
 - For kinetic measurements (more than one measured cycle or interval), choose the range(s) of interest (**Calc. Start** and **Stop**) and the data values from these ranges can be evaluated using a kinetic calculation.

5.4. Shut Down

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- (i) Log off from the computer and leave the instrument switched on
- (ii) Remove any waste e.g. used plates or any other hazardous biological generated during the operation of the instrument must be disposed of as hazardous biological waste either by virkon decontamination (liquids) or autoclaving (solid). Refer to specific Risk Assessments for procedure for disposal of waste appropriately, in accordance with SOP003.

5.5. Spillages inside or on outside surfaces

- (i) If potentially infectious material is spilled inside the microplate reader, disconnect the instrument from the main power supply and remove the USB cable from the connector. Consult the Equipment Manual for the appropriate clean up protocol. Inform the Responsible Person or Laboratory Manager and record in the spill response log.
- (ii) If potentially infectious material is spilled on outside surface, clean the surfaces carefully with cotton wool or soft cloth that has been soaked in 70% IMS. **CAUTION: DO NOT USE VIRKON**

5.6. Equipment Malfunction

- (i) If any part of the equipment fails or malfunctions, contact the LM or RP. With permission of the LM/RP the user should consult the Operator Instruction Manuals to access fault finding, error displays and troubleshooting procedures.

NOTE: If you have any problems / questions regarding the software / the instruments, you should visit the BMG web page <http://www.bmglabtech.com> and read the information on the support page. If you cannot find the answer there contact BMG Labtech using the following e-mail addresses:

- Problems / questions regarding the software: support@bmglabtech.com
- Problems / questions regarding the instruments: tech.service@bmglabtech.com
- On-line bug report form: <http://www.bmglabtech.com/support>

- (ii) All problems and corrective actions **MUST** be recorded in the Equipment Maintenance Log.
- (iii) If the equipment malfunction cannot be rectified according to troubleshooting procedures detailed in the Operator and Users Manuals place a "Do Not Use" notice on the equipment and inform the LM/RP. Contact the manufacturer for advice and coordinate with the LM for external maintenance and servicing.
- (iii) External maintenance and servicing of the equipment can only be performed after it has been suitably disinfected and a 'Decontamination Certificate' issued (a proforma is available on the CBE LEARN). **NOTE:** A BMG disinfection certificate must also be completed before maintenance by BMG personnel or shipping to BMG (Section 4 Operator Manual).

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- (iv) 70% IMS is the only suitable disinfectant that can be used. Only authorised personnel should perform disinfection according to the following procedure:
- Disconnect the instrument from the main power supply
 - Remove the USB cable from the connector
 - Clean all the outside surfaces of the instrument carefully with cotton wool or soft cloth that has been soaked in 70% IMS
 - Place the instrument in a large plastic bag along with the cotton wool / soft cloth that has been soaked in 70% IMS. Ensure that the wool / cloth does not touch the instrument.
 - Close and seal the bag
 - Keep the instrument in the plastic bag for at least 24h
 - After the disinfection time has lapsed, remove the instrument from the plastic bag and clean all outside surfaces of the instrument with cotton wool / soft cloth that has been soaked in 70% IMS
 - Repeat the procedure for disinfection of any accessories, which will be returned with the instrument.
 - Complete the Disinfection Certificate(s).

6. DOCUMENTATION

The following records are outputs of this SOP:

QS-Form-009 Decontamination of Equipment Certificate
Equipment Maintenance Record

These records shall be filed in the Equipment File and stored in the CBE Office or otherwise archived for future review or retrieval.

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SOP Version History

Version Reviewed	Date Revised/ Reviewed	Revision Summary	New Version Number
1.0	01.10.12 P.Hourd	Annual Review – Format revised	2.0
2.0	30/06/2020 J.Bowdrey	Reviewed- no changes.	2.0

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