3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

QUANTUM FLUORESCENCE KITS FOR MESF UNITS OF FITC
Product Numbers **QMF-2 AND QMF-10**
Storage Temperature 2-8 °C. Do Not Freeze

TECHNICAL BULLETIN

Product Description
Quantum Fluorescence Kits for MESF Units of FITC are to be used in the quantitation of fluorescence intensity using flow cytometry. The kits measure Molecules of Equivalent Soluble Fluorochromes (MESF) of FITC in a medium range between 10,000 to 500,000.

MESF units are a relative fluorescence intensity unit which compares a stained sample to the fluorescence intensity of a known solution of equivalent fluorochrome molecules. The kit offers a series of four fluorescence reference standards that have been calibrated against solutions of laser grade fluorescent dye in terms of MESF units per microbead standard, an unstained microbead population and QuickCal data diskette.

The QuickCal software (sold separately) can process data from any cytometer. Using the QuickCal Data Diskette (provided), the program is designed to automatically construct a calibration curve for the specific reagent used and derive MESF values for the cell unknowns. See the attached example of a QucikCal Calibration Report. The calibration data from the Quantum Fluorescence Kits are stored in a database.

Test
The lack of an absolute reference standard for fluorescence intensity has made fluorescence intensity quantitation of unknown samples and calibration of instruments a challenging goal in practical flow cytometry. Quantitative fluorescence standards of the fluorochrome used in sample staining allow for the determination of the fluorescence intensity of the sample in terms of a well defined unit of measurement, such as molecules of equivalent soluble fluorochrome (MESF). Once flow cytometry results in peak (mean) channel number have been converted to MESF units, valid comparison of data from different instruments is possible. In addition, the calibration of the instrument fluorescence intensity scale into MESF units and the calculation of Performance Parameters provide a means of quality control for every instrument run (For definitions and interpretation of Performance Parameters see page 4). The creation and maintenance of a database on the calibration standards and the Performance Parameters in the QuickCal program is an essential tool to instrument quality control and quality assurance over time. The fluorescence intensity of each fluorescence quantitation standard in the Quantum Fluorescence Kit for MESF Units of FITC has been calibrated against solutions of laser grade FITC in units of MESF per microbead. The reference blank is used to measure the threshold fluorescence level of the instrument. Correct use of Quantum Fluorescence Kits for MESF Units allows for:

1. Quantitation of the fluorescence intensity of samples in terms of MESF units
2. Determination of instrument fluorescence threshold
3. Determination of instrument linearity and stability
4. Data comparison over time and between instruments.

MESF and Relative Channel Number
To quantitate fluorescence using a flow cytometer, the peak or mean channel must be expressed as a Relative Channel Number (RCN) on a linear scale. The RCN can be used to relate the instrument settings to the resulting peak channel so that any two channel numbers on the screen, regardless of gains setting and neutral density filter can be directly related to one another. The equation for RCN depends on whether a log or linear amplifier was used to obtain the data:

For a linear amplifier RCN = $\frac{(p) \times (g) \times 10^{(nd)}}{G}$

For a log amplifier RCN = $(p) \times 10^{(nd)}$

Where:

p = peak channel
g = maximum instrument gains
nd = power of the neutral density filter, if no ND filter, nd = 0
G = actual gains setting used.

Once the RCN of each calibrated standard has been calculated, a calibration curve can be constructed and subsequent data can be converted to MESF rapidly and reproducibly. Once flow cytometry results are converted to MESF one may validly compare results from different instruments. If any instrument settings are changed, the relationship between RCN and MESF will be changed, and the RCN for each peak must be recalculated using new values.

Components

M7796	MESF Microbeads - Blank
M6796	MESF Microbeads - FITC Level 1
M6921	MESF Microbeads - FITC Level 2
M7046	MESF Microbeads - FITC Level 3
M7171	MESF Microbeads - FITC Level 4

Physical Characteristics:

Size:	7-10µ
%CV (forward scatter):	< 6.00%
% Singlets:	> 85.00%

Fluorescence Characteristics

MESF	8,000 - 595,000 ± 5,000
%CV	< 15.00%

A Certificate of Analysis containing the specific MESF values for each microbead accompanies each kit.

Q 3254 - QuickCal Data Diskette for MESF

QuickCal Data Diskette containing lot specific information, namely the MESF assignments for each of the five microbead populations, and is provided with every kit. It must be used in conjunction with QuickCal software version 2.1 (sold separately). QuickCal software version 2.1 is available for both Windows 95 and Macintosh platforms. Since most programs run faster when installed on the hard disk of computers, it is recommended that the Q-NXXXXX directory be manually copied from the floppy disk to the hard disk. To open the QuickCal Data diskette, go to the File Menu on the QuickCal software version 2.1 and select Q-NXXXXX directory.

The program will perform a standardized linear regression to construct calibration plots and calculate Performance Parameters of flow cytometers. The program stores this data and calculated unknowns in databases from which Calibration Reports, Sample Quantitation Reports and Quality Control Reports, may be obtained.

Reagents

Microbead standards are suspended at approximately 2×10^6 beads per ml in phosphate buffered solution containing surfactants and 0.1% sodium azide as preservative.

Precautions and Disclaimer

Due to sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

Store Quantum Simply Cellular microbeads at 2-8 °C. Do Not Freeze.
Remove QuickCal Data diskette from shipping package. Allow to warm up to room temperature before use. Store diskette at room temperature.

Procedure

Materials and Equipment Required but not Provided

QuickCal v2.1 Software
Antibodies
Wash buffers, pH 7.4, containing 1% BSA
Erythrocyte lysing solution and fixative
Flow cytometer sheath fluid
IBM compatible computer, color monitor and printer

Procedure

1. Vigorously shake the reagent bottles to ensure that the microbeads are in uniform suspensions.
2. Prepare a dilution of the MESF Microbeads - BLANK by adding 1 drop to 0.5 ml of PBS (phosphate buffered saline, pH 7.2). Fluorescence intensity of the fluorochromes (e.g., FITC) is very sensitive to small changes in pH. Dilution of the MESF microbead standards and the unknown samples in the same diluent is critical.
3. Analyze the Microbeads. Adjust the flow rate so that the count rate is optimal for your instrument.
4. Create a FL1 (FITC) histogram gated on forward scatter vs. side scatter. Place a live gate around the singlet population on the forward vs. side scatter histogram.

5. Verify that the reference blank appears on scale.
6. Prepare a diluted suspension of the MESF Microbead standards by adding 1 drop of each of the four MESF Microbeads (Levels 1 to 4) to the suspension already prepared with the Blank Microbeads.
7. Analyze the Microbeads. Determine the peak channel of each microbead standard. Record the instrument settings (e.g., amplifier gains, PMT, voltages, etc.). When establishing a calibration plot, make no further adjustments to the instrument once data collection has begun.
8. Calculate the fluorescence RCN for each of the quantitative standards, if necessary.
9. Analyze the data using the QuickCal Program for Quantum Fluorescence Kits for MESF Units.

Fluorescence Quantitation of an unknown Sample

10. Maintaining the same instrument settings, analyze the unknown sample diluted in the same PBS diluent.
11. Determine the peak channel of each population.
12. Analyze the data using the QuickCal Program for Quantum Fluorescence Kits for MESF Units.

NOTE: Prepare all suspensions immediately prior to use.

Use of QuickCal Data Diskette

Software Installation

Since most data run faster when installed on the hard disk, it is recommended the the Q-NXXXXX directory (folder) be manually copied from the floppy disk to the hard disk, keeping the diskette as a backup.

Software Operation

To use the QuickCal directory (folder), open the QuickCal v2.1 previously stored on the hard disk (see installation and operating instructions included with QuickCal v2.1 program). The program will always open to a demo database. Under the pull down file menu, choose Select Directory. From the browsers, select and open the specific serial numbered directory corresponding to the populations of standards from which you wish to construct the calibration plot.

Validation

When the QuickCal program opens, a data input screen appears, Figure 1. Before proceeding to a calibration or determination of unknown sample intensity/binding, the user should make sure that the proper kit (name) and correct assigned values have been entered into the input screen of the QuickCal program. This information is found in the Certificate of Analysis that accompanies the product.

Entering Data

Calibration Data Entry

To start the data entry process, click the Data button on the screen if the data acquisition is performed the same day. Otherwise, enter the actual acquisition date using the four-digit year format (e.g., 01/12/1996). Press or type in the requested information and press the enter key after each entry. For the program to perform the linear regression, every field must contain information, with the exception of the optional Inf. field. When the computer requests the channel value of Bead 0, be sure to enter the peak channel of the Certified Blank and not the first population of the Quantum Standards. After the last bead Channel is entered and the enter key is pressed, the program will automatically perform the linear regression, calculate the Performance Parameters and ask whether these results are to be saved in the data base. This provides the operator an opportunity to review the data input and correct any entries prior to storage of the information into the database.

Calibration Report

Once the data is stored, the program automatically proceeds to display the Calibration Report Screen, where the calibration plot maybe viewed, along with the relevant information including the calculated Instrument Performance Parameters and their acceptable ranges for the chosen instrument. See Figure 2.

Sample Report

Samples or unknowns may be calculated by going to the Sample Report. First, choose the Cell Type from the data entry screen, then click on the ID field and enter the sample identifier code followed by the enter key. Next, enter the peak (mean) channel number of the unknown sample followed by the enter key. The MESF or ABC value (depending on the kit being run) will automatically be calculated and the cursor will return to the ID field for the next sample. Results, identified with an * in the new field, can be deleted as long as the Sample Report is active. Additional results may be entered using this calibration plot at other times, however, results previously entered can not be deleted from the sample or unknown databases. See Figure 3.

QC Report

By accessing the QC Report, selected information from the database can be obtained, using the active fields indicated on the screen. Clicking on the Extract Data button will take the selected data and use it to plot six (6) Levy-Jennings plots, as well as perform statistical analysis (mean, standard deviation, min. and max.) on a number of instrument performance

parameters. The report also lists the extracted records and highlights any outliners (*) in red. See Figure 4. For a more detailed explanation of features and functions of the QuickCal program, the operator is referred to Help screens.

Results
Interpretation of Instrument Performance Data

The system performance parameters i.e., Coefficient of Response, Zero Channel Value, R-Sqrd, AveRes%, number of decades and binding threshold, indicate the position of the Window of Analysis and the performance of the instrument with respect to the specific antibody used to label the Quantum Simply Cellular microbeads.

R-Sqrd is the coefficient of determination and is a measurement of the linearity of instrument response to the labeling antibody. However, R-Sqrd is rather insensitive to the log data of the regression. **AveRes%** (average residual percent) is a measure of linearity of instrument response to the labeling antibody and is more sensitive to the log data of the regression. AveRes% should always be less than 3% for MESF Kits.

Log Amp Decades is the calculated number of decades covered by the log amplifier from the slope and the histogram channels. Variations in this number may indicate differences in the antibody or the sample preparation procedure. For a four-decade log amplifier, the acceptable range is 3.7 - 4.3.

Coefficient of Response is the slope of the regression line on the 256-channel histogram scale and is related to the dynamic range (e.g., three or four decades) the instrument covers. This value should be approximately 64 for a four-decade dynamic range and 85.3 for a three-decade dynamic range.

Threshold is the lowest number of MESF units detectable above noise. The threshold should be less than 1000 MESF units.

Zero Channel Value (ZCV) is the MESF value of channel zero. It indicates the position of the Window of Analysis with respect to sample space. Acceptable values for ZCV are <200 MESF for FITC conjugates and <100 MESF for PE conjugates.

Product Profile
Acceptable Range for System Performance Parameters

Parameter*	Range
Av Res %	<3%
Detection Level	<1,000 MESF
Coefficient of Response	59 - 69
(Decades of Log Amp)	(3.7 - 4.3)
Zero Channel Value	FITC <200 MESF
	PE <100 MESF

*four-decade log amplifier

These acceptance ranges are based on somewhat limited data and therefore should be only used as guidelines.

Specificity
Limitations

1. Proper storage (2-8°C) and handling are essential to maintain the calibrated binding capacities of the microbeads.
2. Vigorous shaking of the reagent bottle prior to use is essential to obtain a uniform suspension.
3. The flow cytometer must be properly aligned prior to analyzing the microbeads.
4. Reagent saturation must be maintained for both the cells and the microbeads. Titration assays are recommended.
5. Wash buffer and fixative solutions must be carefully adjusted to pH 7.2 - 7.4.
6. Samples of unknowns and microbead standards must be resuspended in the same pH and ionic environments.
7. Since Quantum.ini files are encrypted, QuickCal Data Diskette can only be used with the QuickCal program or versions of WinList that contain QuickCal functions. In addition, the expiration date in the Quantum.ini files limits the calibration function in the QuickCal v2.1 program. In other words, the fluorescence intensity of new unknown samples may be entered at anytime using any of the calibration plots derived prior to the expiration date of the standards, however, no new calibration plots may be derived from standards after their expiration date.

References

1. Vogt, R., et. Al., Model system evaluating fluorescein-labeled microbeads as internal standards to calibrate fluorescence intensity of flow cytometers. Cytometry, **10**, 294 (1989).
2. Sisken, J., Fluorescent Standards. In: Methods in Cell Biology, Taylor, D., and Wang, Y., (Eds.), Academic Press Inc., San Diego, p30 (1989).
3. Schwartz, A., Instrument Compensation and Calibration methods: Reducing Theory to Practice. Progress in Cytometry. Reports from the Third European Cytometry User's meeting, June 5-7, Belgium (1989).
4. Longobardi Givan, A., Flow Cytometry First Principles, Wiley Liss, New York, p88 (1992).
5. Horan, P., et al., Standards and Controls in Flow Cytometry, In Flow Cytometry and Sorting, 2nd Ed., Melamed, M. et al., (eds.)Alan R. Liss, New York (1990).
6. National Committee for Clinical Laboratory Standards: Clinical Applications of Flow Cytometry: Immunophenotyping of leukemic Cells; Proposed Guideline, NCCLS Document H43-P, NCCLS, Villanova, PA (1993).

lpg 3/99

Figure 1 – Data Input Screen

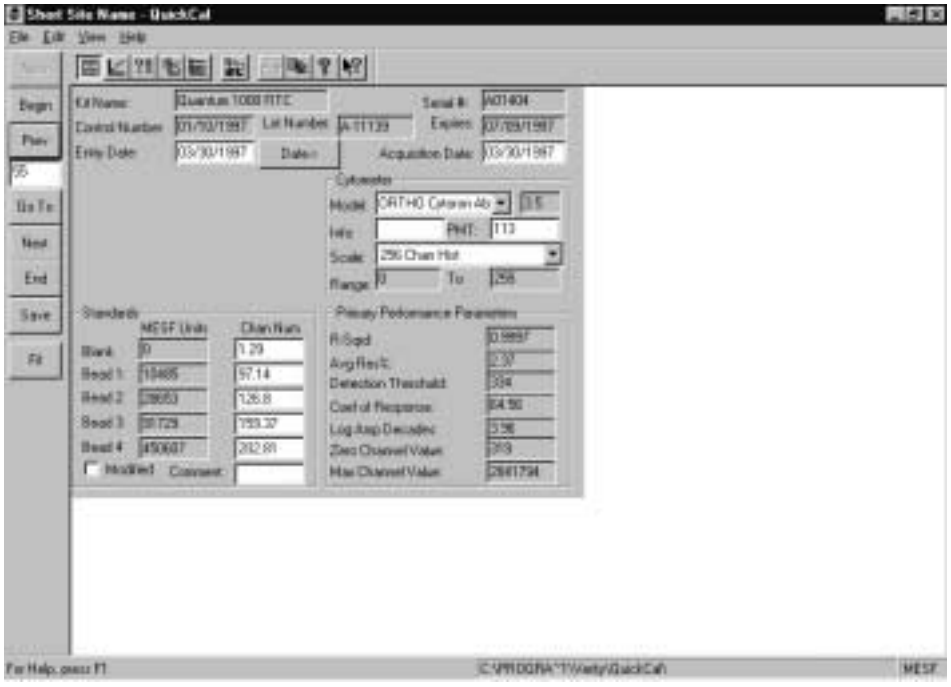

Figure 2 – *Calibration Report*

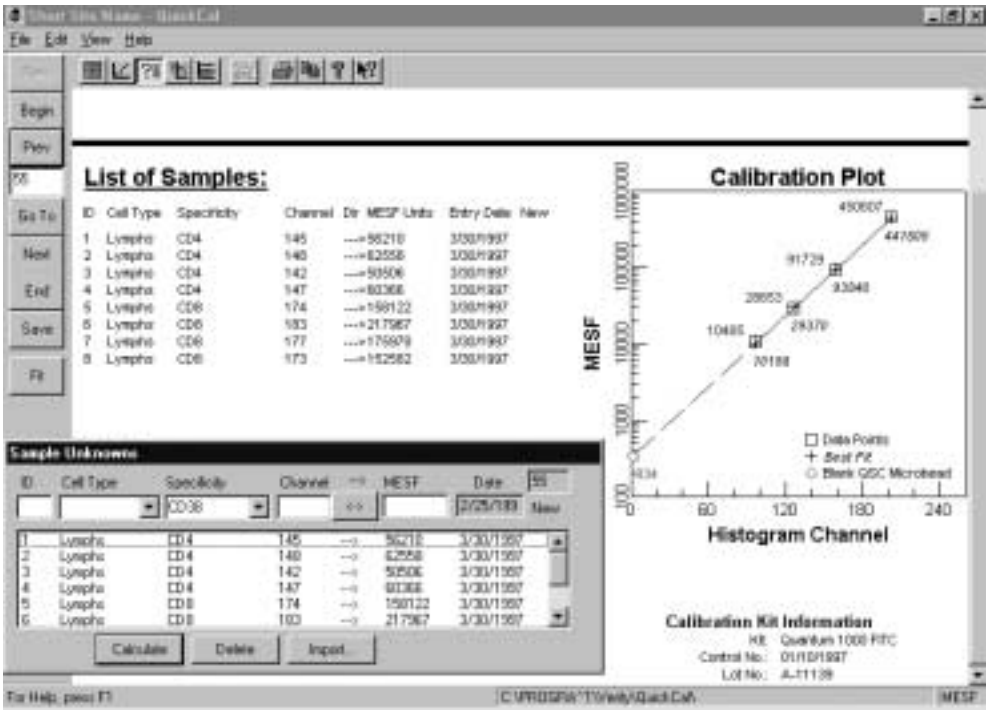

Figure 3 – *Sample Report*

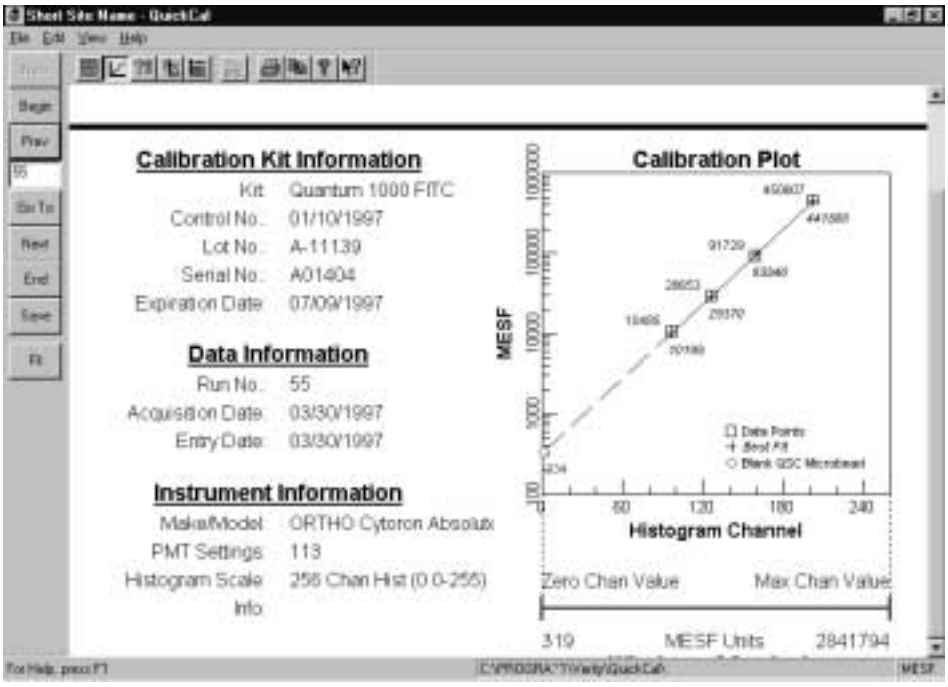

Figure 4 – *Quality Control Report*