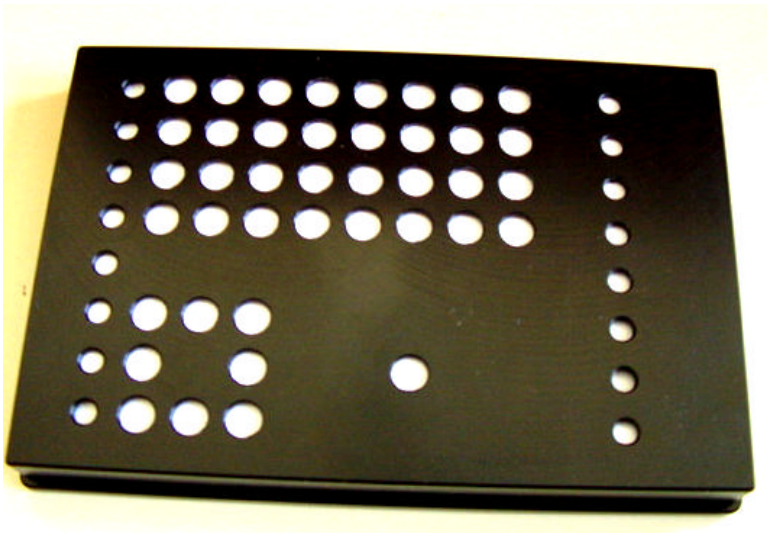
Luminescence QC Pak™



Operating Manual
Version 1.0

Notes:

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Microtiter is a registered trademark of Dynex Technologies.

Microsoft and **Windows** are registered trademarks of Microsoft Corporation.

Intel and **Pentium** are registered trademarks of Intel Corporation.

Warranty

The manufacturer will repair or replace, at its discretion, any product with defects in materials or workmanship within one year of the date of purchase. QC Pak™ units that are outside the warranty period can be re-calibrated at the factory for a small charge.

Neither the manufacturer nor the distributor of this product provides any warranty for the appropriateness of instruments for a particular purpose, and assumes no liability for the results derived from microplate readers.

Contents

Background

Microplates (also called “microtitre,” “microtiter®,” or “micro titration” plates) provide a rapid way to measure the luminescence, absorbance, fluorescence, etc. of a large number of samples. The volume of a sample is typically about a few hundred µL, and a large number of samples can be analyzed in a short time. A 96 well plate is commonly used for these analyses, and the plate can contain samples, blanks, controls, and standards.

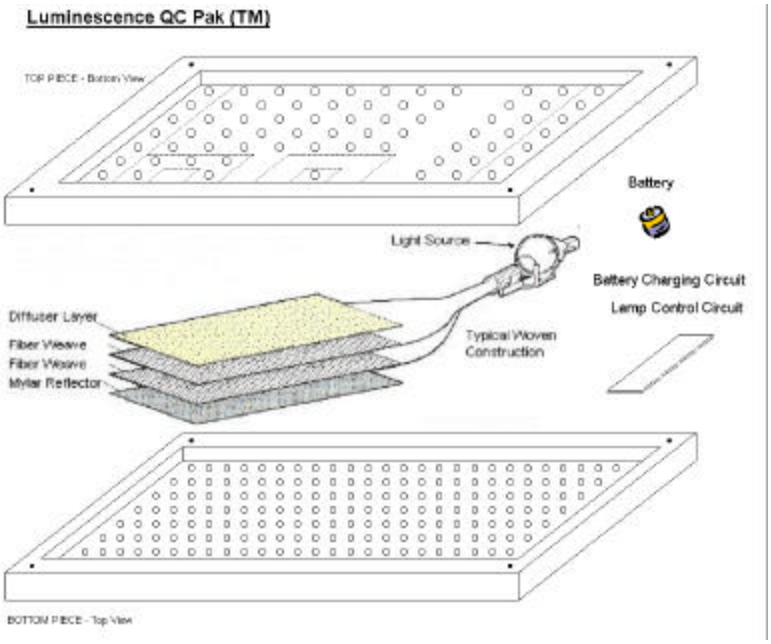
A broad variety of analytical measurements can be performed in microplates including absorbance (OD), luminescence (L), fluorescence intensity (F), time-resolved fluorescence (TRF), and fluorescence polarization (FP). In each case, the fundamental measurement is the same as when a standard cuvette is used, but the instrument (called a “reader”) is capable of reading the entire plate is less than a minute and generate a complete report.

While the general design of a reader is identical to that of a cuvette system (e.g. an absorbance reader is similar in nature to a spectrometer), the optics are configured such that 96 samples can be read on a plate with an 8 x 12 format.

Introduction

The Luminescence QC Pak™ consists of:

1. a Luminescence Standard Plate
2. an Evaluation Software Template
3. a 9V battery charger
4. “Short Installation Instructions”
5. “Certificate of Validation”



The Luminescence Plate consists of a microplate housing, a battery, a charging circuit, 2 light-emitting diodes (LED’s), a mesh of fiber optics woven into a screen, a reflective panel, a diffuser, and pattern of masks and filters to regulate the light.

The Excel Template calculates a variety of parameters such as linearity, crosstalk between wells, and alignment of the plate in the reader. A report is automatically generated which can be printed and/or archived.

This manual describes the design, installation, and use of Innovative Instrument’s Luminescence QC Pak. In addition, it includes a glossary of commonly used terms for luminescence measurements.

Getting Started

Unpacking Fluorescence QC Pak

The following items are included in *Luminescence QC Pak*:

- A test microplate
- A CD ROM
- A battery charger
- Short Installation Instructions

If any component of the shipment is missing or damaged, please contact your shipping company and Innovative Instruments, Inc.

We suggest that you fill in the information below for quick reference.

Reader Serial Number:	_____
QC Pak™ Serial Number:	_____
Software Version:	1.0
Manual Version:	April 29, 2004
QC Pak™ Version:	1.0
Date Purchased:	_____

Computer Configuration

The computer requirements for QC Pak are:

- Microsoft® Windows® 98 or higher
- Intel® Pentium® 75 MHz or faster
- 128 MB RAM or greater
- 100 MB Hard Disk or larger
- Excel 97 or newer

Installing the Application Software

To install the Applications software:

a) Place the CD containing the software into your CD drive.

b) Use Microsoft® Explorer to locate *Setup.exe* on the CD and run Setup.

c) A series of dialog boxes will be presented that lead you through the installation program. Follow the instructions of each dialog box. We recommend that you accept all default settings (e.g. directory options).

d) Activate the software by opening Excel and selecting the spreadsheet, which will be in the directory in which it was installed.

Using QC Pak

Charging the Plate

a) The Luminescence Test Plate is shipped fully charged and ready to use. The fully charged Luminescence Test Plate will emit light for about 50 hours before recharging is needed.

b) To recharge the plate, connect the 9 V AC power supply (included) to a wall socket.

c) Verify that the Luminescence Test Plate is switched Off, and connect the 9 V plug.

d) Charging is underway if the green LED between the power connector and the power switch blinks.

e) Charging is complete when the LED stops blinking and remains lit, normally within 2 hours.

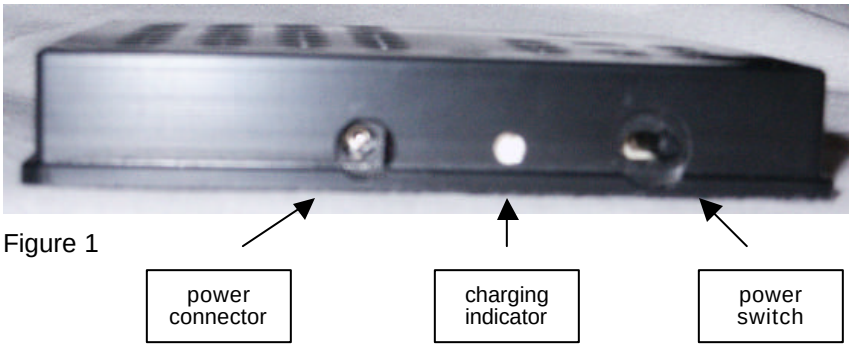


Figure 1

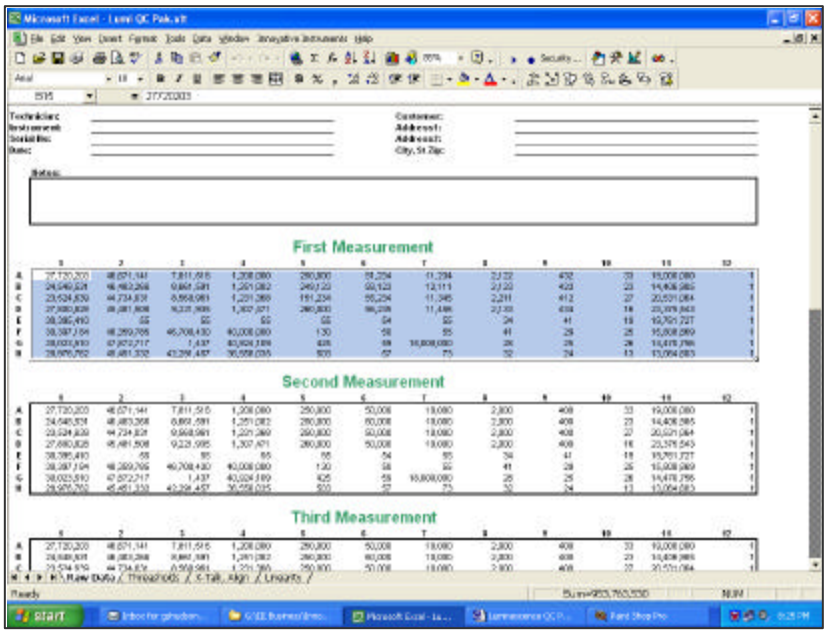
Charging Conditions:

- temperature limit during charge 0° C to 40°C
- temperature limit during discharge -30° C to 50° C
- temperature limit with no current -40° C to 55° C
- lifetime when stored at 0° C to 35 C° 3 to 6 years
- lifetime when stored at 35° C to 50 C° about 2.5 years

Collecting Data

a) Switch on the Luminescence Test Plate using the "toggle" type power switch (figure 1).

b) Place the microplate in your reader. The controls in figure 1 should be oriented toward the left side of the reader. A detailed discussion of the plate is presented in Appendix A.



c) Set the measurement time to 1 second. If your reader has manual "Gain" setting, it should be set to "Medium" or "Low." If your reader calculates Gain automatically, it should calibrate on well A2.

d) Read the plate five times.

e) Copy the data into the "Raw Data" sheet of the QC Pak analysis software using the "First Measurement" to "Fifth Measurement" data areas. (Figure 1).

Figure 2: Raw Data tabViewing the Results

The analytical data can be viewed by selecting the tabs on the bottom of the spreadsheet.

The Crosstalk and Alignment Tab

The Crosstalk and Alignment tab presents:

a) Averaged RLU

The averages of the five measurements are indicated in Figure 3. The green cells indicate wells that should exhibit luminescence, and the gray cells indicate background from empty wells on the plate.

Luminescence Averages

	1	2	3	4	5	6	7	8	9	10	11	12
A	27,720,203	46,671,141	7,811,516	1,200,000	250,000	50,247	10,247	2,024	406	33	19,000,000	1
B	24,548,531	46,483,266	8,661,591	1,251,082	249,825	59,625	10,422	2,025	405	23	14,406,985	1
C	23,524,639	44,734,031	8,560,961	1,231,368	230,247	51,047	10,269	2,042	402	27	20,531,064	1
D	27,800,828	45,481,508	9,221,935	1,307,471	260,000	51,247	10,291	2,027	407	16	23,375,543	1
E	30,395,410	55	55	55	55	54	55	34	41	19	19,761,727	1
F	30,397,184	48,359,785	46,700,430	40,000,000	130	50	55	41	29	25	15,808,869	1
G	30,023,510	47,872,717	1,437	40,924,109	425	59	18,000,000	28	25	26	14,470,756	1
H	28,976,762	45,451,332	42,291,457	36,550,035	503	57	73	32	24	13	13,064,603	1

Figure 3: Averaged Data

b) Microplate Alignment Plot

The Microplate Alignment Plot (Figure 4) presents the luminescence readings for Column 1 and Column 11 for rows 1 - 8. The cells in column 1 and column 11 of the plate have about the same intensity and are approximately parallel, if the tray is properly aligned.

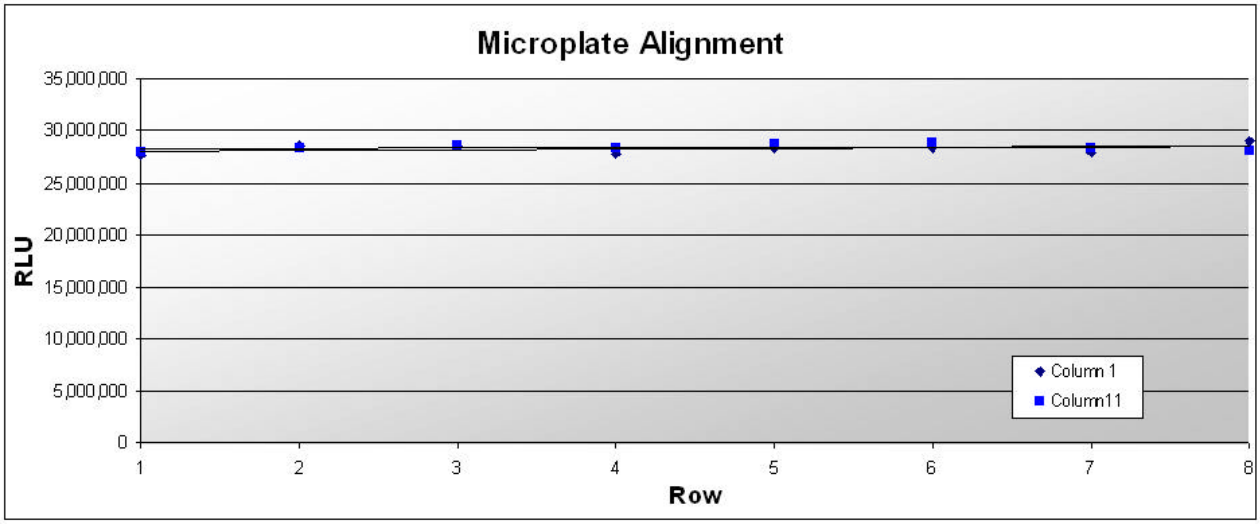


Figure 4: Microplate Alignment Plot

c) Alignment and Cross-Talk Data and Results

The Alignment and Cross-Talk Results (Figure 5) describe the statistical analysis of the data and presents the results for the Alignment, Cross Talk, and Signal to Noise tests.

Alignment	
Mean Column 1	28,298,383
Mean Column 11	28,427,443
Standard Deviation (p) Column 1	395371
Standard Deviation (p) Column 11	270951
% STD (p) Column 1	1.40%
% STD (p) Column 11	0.95%
Slope Column 1	75,988
Slope Column 11	23,699

Cross-Talk	
Mean Blank	29.8
Best Case Positive	18,000,000
Best Case Background	49.4
Percent Crosstalk	1E-06
Worst Case Negative	1437.0
Worst Case Surrounding	43,518,733
Percent Crosstalk	3.23E-05

Results:

Alignment Column 1:	Reader Passes!
Alignment Column 11:	Reader Passes!
Cross-Talk, Best Case:	Reader Passes!
Cross-Talk, Worst Case:	Reader Passes!
Signal to Noise (Col 8):	9 Reader Passes!

Figure 5: Alignment and Cross-Talk Data and Results

The acceptance criteria are:

- Alignment Column 1 – slope is less than 1.0 %
- Alignment Column 2 – slope is less than 1.0%
- Alignment Left to Right – the average 1 & 2 within 5%
- Crosstalk, Best Case – less than 3×10^{-5}
- Crosstalk, Worst Case – less than 1×10^{-2}
- Signal to noise – less than 3

The Linearity Tab

The Linearity Tab presents:

d) Averaged RLU

The averages of the five measurements are indicated in Figure 6. The green cells indicate wells that should exhibit luminescence, and the gray cells indicate background from empty wells on the plate.

Luminescence Averages

	1	2	3	4	5	6	7	8	9	10	11	12
A	27,720,203	46,671,141	8,011,516	1,200,000	250,000	50,247	10,247	2,024	406	33	28,000,000	1
B	28,548,531	46,483,266	8,661,591	1,251,082	249,825	59,625	10,422	2,025	405	23	28,406,985	1
C	28,524,639	44,934,031	8,560,961	1,231,368	250,247	51,047	10,269	2,042	402	27	28,531,064	1
D	27,800,828	45,481,508	9,221,935	1,307,471	260,000	51,247	10,291	2,027	407	16	28,375,543	1
E	28,395,410	55	55	55	55	54	55	34	41	19	28,761,727	1
F	28,397,184	48,359,785	46,700,430	40,000,000	130	50	55	41	29	25	28,808,869	1
G	28,023,510	47,872,717	1,437	40,924,109	425	59	18,000,000	28	25	26	28,470,756	1
H	28,976,762	45,451,332	42,291,457	36,550,035	503	57	73	32	24	13	28,064,603	1

Figure 6: Averaged Data

a) The Linearity Plot

The Linearity Plot (Figure 7) shows the regression line of the averages of the 4 replicates, for each of the 8 luminescence intensity standards. The Y-axis represents the measured RLU from the reader, which are "cut and pasted" into the Raw Data tab, and the X-axis represents the factory-calibrated standards, or "Nominal Values."

By default, the RLU from the reader are normalized to a scale of 100,000. If desired, you can change the "**Normalize to**" function to adjust the scale from 1 to 100,000,000 in steps of 10 using the arrows to the left. This function is a multiplier to obtain an appropriate dynamic range.

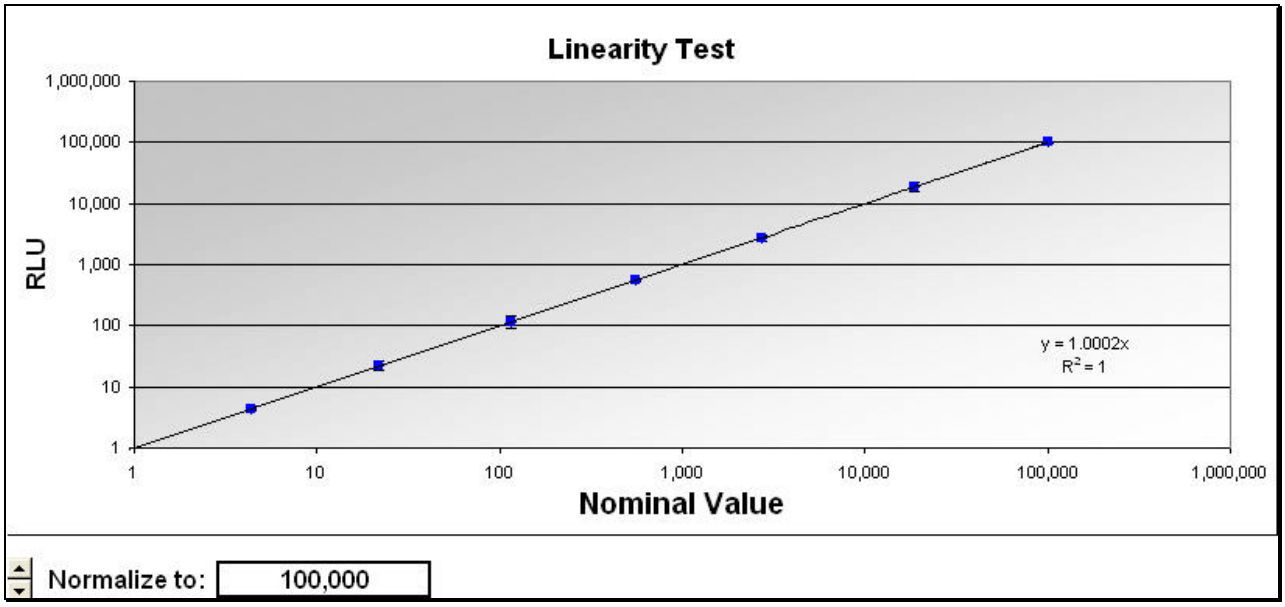


Figure 7: The Linearity Plot

b) The Data Region

The Data Region (Figure 8) presents a summary of the analytical data. The values are dependent on the selected number for the **Normalize To** value; and you should select the Normalize Value to one where all values in the blue field are finite and non-zero.

"**QC Pak Mean**" shows the averages of the raw RLU measurements from the test reader, "cut and pasted" into the Raw Data tab.

"**QC Pak Std Dev**" shows the calculated Population Standard Deviations of the raw RLU values.

"**Normal Mean**" shows the raw RLU averages normalized to the "**Normalize to:**" value.

"**Nominal Mean**" shows the factory calibrated "gold standard" RLU averages, for a given plate.

"**Normal Std Dev**" shows the QC Pak Std Dev on the normalized scale.

"**3 x Std Dev**" shows the "Normal Std Dev" multiplied by 3, which are used in the Linearity plot error bars.

QC Pak Mean	QC Pak Std Dev	Normal Mean	Nominal Value	Normal Std Dev	3 x Std Dev
45892487	742102	100,000	100,000	1617.0	4851.1
8614001	473722	18,770	18,681	1032.2	3096.7
1247480	39134	2,718	2,721	85.3	255.8
252518	4333	550	551	9.4	28.3
53041	4176	116	115	9.1	27.3
10307	633	22	22	1.4	4.1
2029	61	4	4	0.1	0.4
405	11	0.9	0.9	0.0	0.1

y = mx + b	
Slope =	1.0000
Y int. =	10
r^2 =	1.00000

Figure 8: The Data Region

c) The Results Field

The Results field (Figure 9) indicates the results of the various tests.

Results:

Linearity Relative to Nominal:	Reader Passes!
Same-Well Precision (High):	Reader Passes!
Same-Well Precision (Low):	Reader Passes!

Figure 9 The Results Field

The acceptance criteria are:

- Linearity Relative to Nominal - R^2 should be greater than 0.990
- Same Well Precision (High Concentration) - should be less than 2%
- Same Well Precision (Low Concentration) - should be less than 10%

Appendix Description of the Microplate

The microplate should be placed so that the wells containing the red fluorophore are oriented to the right side of the reader.