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TR717™ Microplate Luminometer

Instructions for Unpacking, Setup, Verification, and 96- to 384-Well Conversion

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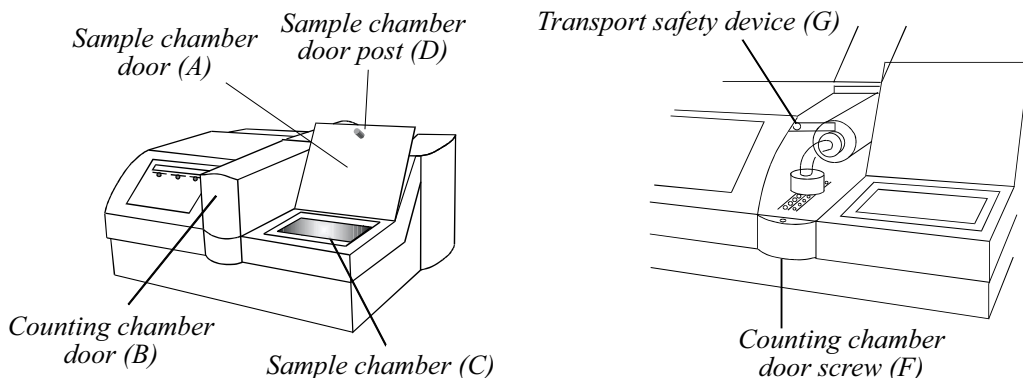
I. TR717™ UNPACKING & HARDWARE SETUP

These instructions must be followed to insure proper operation. Do not omit any items. It is recommended that all packaging be saved in case the instrument needs to be shipped or moved at a future date. Do not plug in the instrument until these instructions have been completed.

1. Carefully remove the instrument from its shipping box, place it on the bench where it will be used, and remove any plastic or bubble wrapping.
2. Open the sample chamber door (see Figure, marker "A"). Using the screwdriver provided, loosen the counting chamber door screw (F; underneath the counting chamber door, B) and open the counting chamber (B) to access the safety transport device (G).
3. Loosen the thumbscrew on the transport safety device (G; located to the left of the counting chamber, on the black panel that separates the front and back of the instrument), slide the transport device to the left (away from the PMT) and re-secure the screw tightly.
4. Close the counting chamber door and tighten its screw.
5. Remove the empty microplate from the sample chamber (C). Remove the blue/white cylinder from the sample chamber door post (D).
6. Attach the reagent bottles (if desired), and attach the waste line to waste port on the back of the instrument. Place the black metal cover over the fan on the back of the instrument.
7. Attach the control cable to the available (top) 25-pin port on the back of the instrument, and plug the power cord into its receptacle near the instrument power switch.

NOTE: After unpacking and setup, the sample chamber door may not close properly if the door lock has been engaged (two brass bars will be visible in the hole in which the sample chamber door post is positioned when the sample chamber door is closed). To reset the door lock:

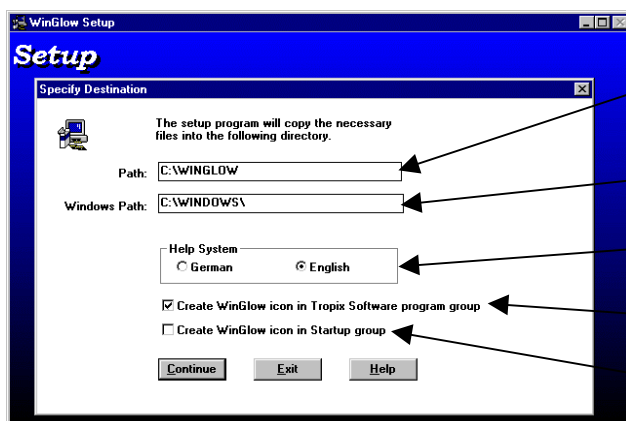
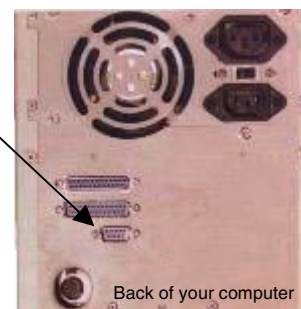
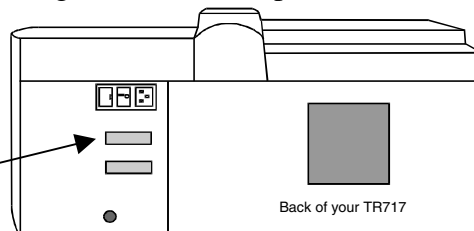
1. Prepare a short WinGlow "Integrate" protocol (measure only 1 well for 1 sec).
2. Depress the switch in the bottom of the hole for the sample chamber door post.
3. While depressing the switch, start the WinGlow protocol.
4. After the protocol has run, the door lock should reset and the door should shut.



II. COMPUTER SETUP AND WINGLOW INSTALLATION

Please read the TR717™ Instrument Operation and Software Guide thoroughly before using your TR717™. It will provide a comprehensive understanding of instrument operation as well as an introduction to WinGlow.

1. Place your PC next to the TR717™.
2. Attach the 25 pin end of the enclosed cable into the top port on the back of the TR717™.
3. Attach the 9-pin end of the serial cable to the "COM1" serial port on the back of the computer. Alternatively, if you need to use another COM port that has a 25-pin receptacle, use the 9-pin to 25-pin adapter provided.
4. Insert the WinGlow diskette into the A:/ drive on your computer, and Run the [setup.exe] program. The default choices that you are presented with in the setup window are standard. Click "Continue" to finish installation.



"Path" represents where the program files will be stored on your hard-drive. The default setting will create a new folder called "WinGlow". Change the path if you want the program files saved elsewhere.

"Windows Path" is the location where windows configuration files will be saved. Except in special cases, leave this path unchanged.

You have the choice of either English or German on-line help files.

A check in this box will create a WinGlow icon in a Tropix program group (Windows 3.x) or a icon/group in the start task-bar (Windows 95)

A Check in this box will allow WinGlow to start every time your computer is turned on.

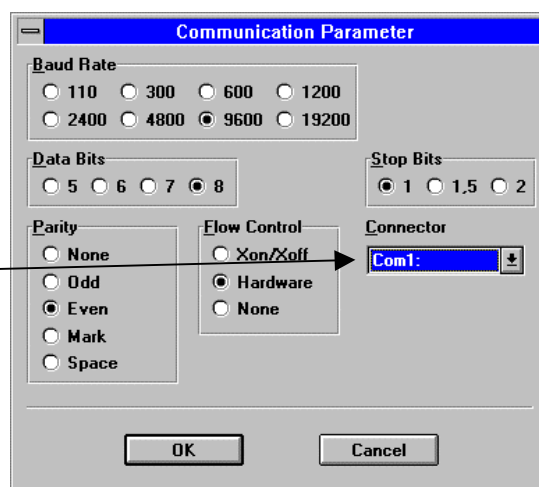
5. In most cases, WinGlow will configure itself. In some cases, however, it may be necessary to change some communication settings before the PC can "talk" to the TR717™. You can get to this window by starting WinGlow, going to the "system" menu and selecting "communications".

In most cases, you have attached your computer to your TR717™ through the COM1 port on the back of your computer. The default settings of this panel are okay.

If you are using a communications port on your computer other than COM1 (i.e. COM2) select that port in the "Connector" box.

If you are unsure as to what COM port you are using, refer to your computer's users guide or call your computer supplier's helpdesk.

Do not change any other item in this panel. The communication parameters are set specifically for the TR717™.



III. VERIFICATION WITH THE STARTUP CHEMISTRY KIT

The TR717™ Startup Chemistry Kit is intended for use in software training and to verify that the instrument is measuring properly. The kit is designed to provide familiarity with the TR717™ and to establish a baseline of instrument performance. The kit is not intended to "calibrate" the instrument; due to the nature of luminescence and luminometers, calibration is not required.

A. Startup Chemistry Kit Components

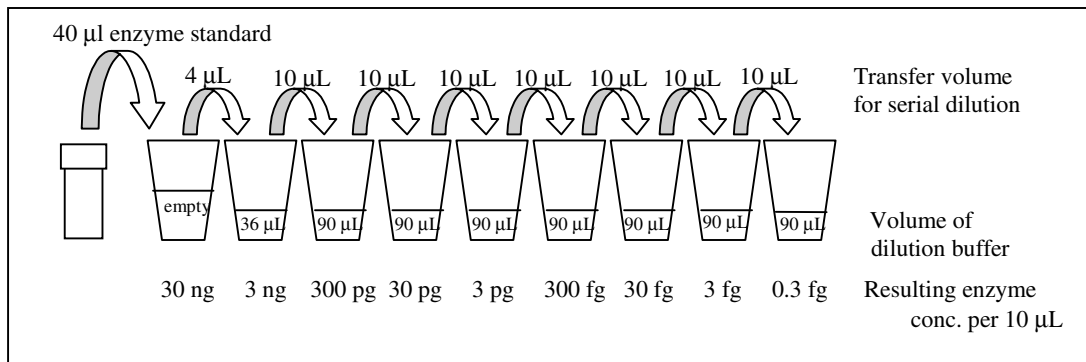
The Startup Chemistry kit contains reagents sufficient for 250 assays. All components, with the exception of the microplates, should be stored at 4°C.

Component	Cat. No.	Size
CSPD® Ready-to-Use with Sapphire-II™ Enhancer	CD025RX	25 mL
Alkaline Phosphatase Standard	-	200 µL
5X Enzyme Dilution Buffer	-	5 mL
Tropix 96-well Microplate	TMP100	5 plates

B. Preparation of an Alkaline Phosphatase Dilution Series

All assays should be performed in triplicate.

1. Dilute 1 mL of 5X Enzyme Dilution Buffer with 4 mL of ddH₂O prior to use. Alternatively, 20 mL of ddH₂O may be added to the entire 5X concentrate in the amber vial to produce a 1X stock. Be sure to mark this concentration change on the vial label.
2. Label 9 microfuge tubes for serial dilution of the concentrated enzyme.
3. Leave the first tube empty, aliquot 36 µL of 1X Enzyme Dilution Buffer into tube 2, and aliquot 90 µL into each of the remaining tubes 3-9 (see figure below).
4. Add 40 µL of the Alkaline Phosphatase Standard into tube 1 and discard tip.
5. Transfer 4 µL from tube 1 to tube 2, mix well, and discard tip.
6. Transfer 10 µL from tube 2 to tube 3, mix well and discard tip. Continue the serial dilution in this manner for the remaining microfuge tubes, using a fresh pipette tip for each transfer.



C. Chemiluminescent Detection Procedure

1. Aliquot 3.5 mL of Ready-to-Use Substrate Solution into a separate container and allow to equilibrate to room temperature.
2. Add 10 μ L from each Alkaline Phosphatase dilution (starting with tube 1) to a Microplate well, starting with A1 and moving to the right for each subsequent sample. Perform in triplicate (see figure below).
3. Add 10 μ L of 1X Enzyme Dilution Buffer to wells A10, B10, and C10 to serve as a negative control (see below).
4. Add 100 μ L of Ready-to-Use Substrate Solution to each well and incubate the Microplate at room temperature for 30 min.
5. After incubation, plate is ready to be used in the TR717™. Light emission will peak at 30 min, but will persist for up to several hours.
6. Measurements may be made for as little as 0.1 sec/well, or for as long as 10 sec/well. However, it is likely that measurement for 10 sec will cause an over-saturation of the instrument detectors resulting in an out-of-range error. This will not damage the instrument, but it may take 15-20 hours for the detectors to recover completely.
7. When plotted on a log:log plot, enzyme amount versus Relative Light Units should be nearly linear between 3 ng and 3 fg of enzyme.

	1	2	3	4	5	6	7	8	9	10
A	30 ng	3 ng	300 pg	30 pg	3 pg	300 fg	30 fg	3 fg	0.3 fg	neg
B	↓									↓
C	↓									↓

IV. CONVERSION BETWEEN 96- & 384-WELL MICROPLATE FORMATS

A. Summary Flowchart for Switching from 96- to 384-Well Plate Formats



WinGlow is now ready for running or creating 384-well plate protocols.

B. Notes on 384-well Measurements

1. The instrument is factory set for 96-well plate format.
2. Injection in 384-well mode is disabled. However, the injector tips do not need to be removed when switching from 96- to 384-well mode.
3. Only INTEGRATE protocols can be operated in 384-well-mode. Kinetic and repeated protocols are unavailable in 384-well plate mode.
4. You must use separate protocols for measurements in 96-well and 384-well plate format. It is recommended that you save 96- and 384-well protocols in separate folders.

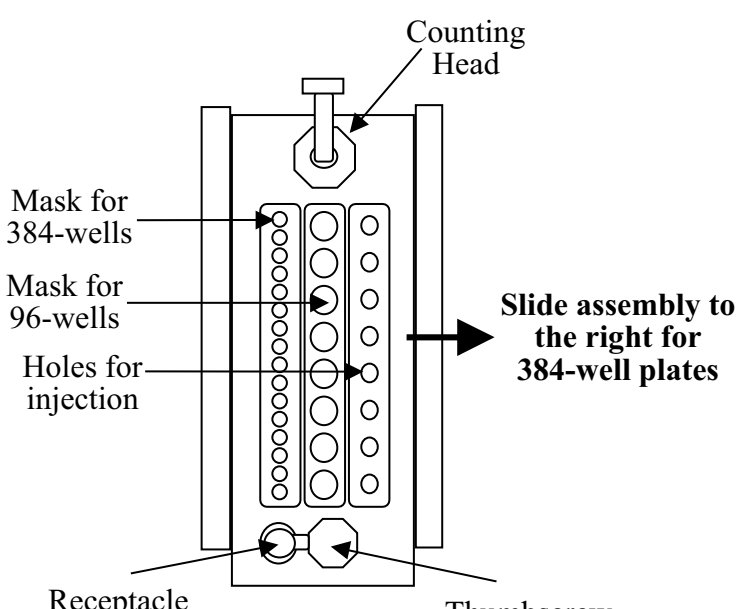
C. Changing Plate Format

WARNING!

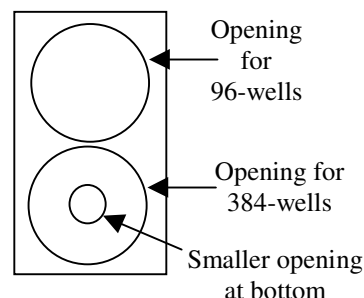
The TR717™ must be **SHUT OFF** before continuing. Failure to do so may cause damage!

WARNING!

Do not slide the mask assembly without lifting the counting head; this may cause damage!

1. Using the screwdriver included with the TR717™, open the counting chamber door.
2. Loosen the thumbscrew multiple turns.
3. While lifting the counting head, slide the mask assembly completely to the right. The assembly is in the correct position when the thumbscrew lines up directly with the recessed receptacle underneath the.

4. When the mask assembly is in the correct position, retighten the thumbscrew. The mask will correctly align itself as the thumbscrew is tightened. Do not over-tighten the thumbscrew; finger-tight is sufficient.

5. While the counting chamber door is open, change the counting head foot (the small black plastic block between the counting head and the mask). The foot provides masking of extraneous signal and a means by which the counting head can slide in the mask.
6. Flip the orientation of the foot so that the smaller opening for 384-wells is oriented towards the bottom and facing towards the back of the instrument.
7. Set the counting head into the opening on the foot with the smaller bottom opening. The other opening will protrude from the front of the counting head. The counting head must be sitting properly in the foot for optimal performance.
8. Close the counting chamber door and re-secure with the screwdriver.
9. The instrument firmware must now be changed. Using the keypad on the front of the TR717™ under the LCD display, select the commands as indicated (commands are selected by pushing the "soft-key" directly underneath the selection on the LCD):



Key-Pad Display			Select	
READY			other	
MEASUREMENT	DIRECTORY	<u>other</u>		
READY				
PROTOCOLS	SET TEMP	<u>other</u>	other	
READY	<u>SETUP</u>	other	SETUP	
REAGENT				
SETUP OPTIONS	<u>INSTR-PARAM</u>	RS232-LINE	INSTR-PARAM	
DATE/TIME				
rlu-FACTOR				
NUMBER IN RANGE	0.00	100.000	enter
INSTRUMENT ID			enter	
ENTER STRING UP TO 4 CHAR.				
INJ. P AVAILABLE			enter	
NO	YES			
INJ. M AVAILABLE			enter	
NO	YES			
INJ. Q AVAILABLE			enter	
NO	YES			
PLATE FORMAT			384	
96	<u>384</u>			
INPUT CORRECT			YES	
NO	YES			

You are now ready for reading in 384-well mode.

D. Resetting WinGlow for 96- to 384-Well Format

When switching well formats from 96- to 384-well (or vice-versa), the end-user needs to instruct WinGlow to recognize the well format that it should be using.

There are two separate ways to set WinGlow for 384-well measurement:

Through WinGlow - Simple Method

1. Make the appropriate hardware changes (in the previous section).
2. Launch WinGlow.
3. Load a protocol with the well format that was just changed from (if switching to 384-well mode, run a 96-well protocol).
4. At the error message (alerting you that the protocol picked cannot be used) click "OK".
5. WinGlow will now reset itself in the appropriate well format. You can now load a protocol for the well format that you just setup, or, if you begin to create a new protocol, WinGlow will only give you a choice for the new well format

By Changing the [winglow.ini] File - Advanced Method

Using this method, it is possible to create major bugs in your computer operating system. This method is only recommended for users very experienced with the "Windows" environment.

1. Launch Windows Explorer (Windows 95/98) or File Manager (Windows 3.1).
2. Open the "Windows" folder located on the C:/ drive.
3. Open the [winglow.ini] file by double-clicking on it (this should run Windows Notepad). The section of the file to change is:

```
[Integrate]
Ratio = 0
Wells = 96
```

4. Change "Wells = 96" to "Wells = 384".
5. Save the file and close notepad. WinGlow is now set to measure 384-well plates.

REMINDER!

1. You will need to create separate protocols for 96-well and 384-well plates.
2. You can only use INTEGRATE measurement protocols in 384-well mode
3. You cannot use injection in 384-well mode

E. Conversion from 384- to 96-Well Format

To switch back to the 96-well plate format, you will basically perform the same operations as described in Sections C & D above, with certain obvious differences:

Section C, Step:

3. While lifting the counting head, slide the mask assembly completely to the **left**. The assembly is in the correct position when the thumbscrew lines up directly with the recessed receptacle underneath the **96-well** mask.
6. Flip the orientation of the foot so that the smaller opening for 384-wells is oriented towards the bottom and facing towards the **front** of the instrument.
7. Set the counting head into the opening on the foot with the **larger** bottom opening. The other opening will protrude from the front of the counting head. The counting head must be sitting properly in the foot for optimal performance.
9. The instrument firmware must now be changed:

Key-Pad Display	You Select
PLATE FORMAT <u>96</u> 384	96 enter
INPUT CORRECT NO YES	YES

You are now ready for reading in 96-well mode.

Section D, Step:

Through WinGlow - Simple Method

3. Load a protocol with the well format that was just changed from (if switching to **96**-well mode, run a **384**-well protocol.
4. At the error message (alerting you that the protocol picked cannot be used) click "OK".
5. WinGlow will now reset itself in the appropriate well format.

By Changing the [winglow.ini] File - Advanced Method

3. Open the [winglow.ini] file by double-clicking on it. The section of the file to change is:

```
[Integrate]
Ratio = 0
Wells = 384
```

4. Change "Wells = **384**" to "Wells = **96**".
5. Save the file and close notepad. WinGlow is now set to measure 384-well plates.

V. Troubleshooting

Problem	Solutions
I just finished TR717™/WinGlow installation and setup. When I started WinGlow to create a new protocol for the first time, it is set for a 384-well template even though the instrument is configured for 96-well mode.	<p>To get the TR717™ into a 96-well plate mode you will need to reset WinGlow. This is done as follows:</p> <ol style="list-style-type: none"> 1. Go to the protocol menu and select "integrate". 2. Click the "new" button. 3. In the 384-well template that appears, select 1 well. 4. Click on "OK" at the bottom right of the screen. 5. Select a filename and save the protocol. 6. Go to the "measure" menu and select integrate. 7. Load the protocol you just created by double clicking on it. 8. You will receive an error message, click "OK". 9. WinGlow will reset and should now be in 96-well mode.
When creating a new protocol, the software is giving options for more injectors than are installed on the TR717™.	When WinGlow is run for the first time, it must synchronize (or initialize) with the TR717™. However, before a protocol has been run, WinGlow runs with default parameters, one of which is for 3 injectors. Once you have run a protocol, WinGlow will synchronize to the instrument, and only parameters appropriate for your instrument will be enabled.
When running a measurement, the instrument makes no noise and unexpected data is reported by WinGlow. Or, I cannot get the TR717™ to respond to software commands.	WinGlow is in simulation mode. Go to the "system" menu and select "system options". Deselect the simulation mode option.
When trying to run my first measurement, an error message "instrument off-line, do you want to continue" appears.	<ol style="list-style-type: none"> 1. Confirm that the cable is connected to the correct port on the TR717™ and the COM port on the back of your computer. 2. Check the communication parameters within the "system" menu in WinGlow to ensure that the correct COM port has been selected. 3. If you loaded a measurement protocol within a few sec of starting WinGlow, try again. It may take a few sec for WinGlow to recognize the TR717™ and initialize.

Problem	Solutions
After switching from 96-well to 384-well plate format, I ran a plate and all the data was "0's".	During the instrument firmware conversion (Section IV-C, Step 9), the "rlu-FACTOR" was probably reset to "0". Go through the firmware setup again, paying extra attention to the setting for "rlu-FACTOR"; if it is now "0", reset it to "1".
When I do an "Excel Evaluation" from WinGlow, Excel is not activated, or the "Lumi" macros do not appear in Excel.	Before doing an "Excel Evaluation", the WinGlow data file must be saved to a disk, hard drive, or network folder. Excel must be open before initiating "Excel Evaluation". If the data file has been saved and Excel is open, but "Lumi" macros do not appear, it is possible that the Lumi macro file [lb96p.xlm] has been corrupted. This can happen when Excel is closed; a dialog box appears asking if you would like to save changes to [lb96p.xlm]. Always answer "no" to this question; a "yes" answer will cause the file to be overwritten, which will corrupt the file so that next time an "Excel Evaluation" is performed, the Lumi macros will not be properly inserted into Excel. The simple solution to this problem is to re-install WinGlow on your computer, a 2-3 min procedure.
Something is wrong with my TR717™ or WinGlow and I am not sure what the problem is.	Contact Tropix Technical Service by phone (800-542-2369 within the U.S. or 781-271-0045 outside the U.S.) or email (tropix@appliedbiosystems.com).

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