Instructions for Using Molecular Devices LMax Luminometer

Before you begin, check that an empty 96-well microplate* is in the machine and make sure you have the following items with you:

1. 50 ml conical tube with ddH_2O (at least 15 ml).
2. 50 ml conical tube with 70% EtOH (at least 15 ml)

} For washing injector tubes

3. Your lysates in a 96-well, white, opaque microplate.
4. Luciferase Assay solutions, protected from light, in 15 ml conical or snap cap tube (*you need an extra 2 ml to prime the injector tubes).*

Notes:

*** The Lmax must always have an empty plate inside to avoid accidentally reading or priming injectors and allowing liquid to get inside.**

**** You must make sure that the plate you are to read does not have any moisture/condensation underneath. Any liquid that gets inside the luminometer must be <u>immediately</u> cleaned up!**

***** You absolutely <u>MUST</u> wash the injector tubing when you are done reading your plate(s)!**

TO READ:

1. Turn on the Lmax (switch is directly above the power cord on the right side of the back of the machine).

2. Open the program you want to use: eg "Luciferase" or "Dual Luciferase"

3. Specify instrument settings (YOU MUST COMPLETE ALL STEPS PRIOR TO READING YOUR MICROPLATE).

a. Choose **Instrument Setup**… from the **Control** menu OR click the **Setup** button in the toolbar of the active, expanded plate section.

b. From the left column, select and specify **Integration** and **Pre-measurement** times in the right column (For single luciferase, generally, 15 sec is ok and no pre-read is necessary. For dual luciferase, use 2 sec premeasurement delay and 10 sec measure).

c. Select **Injection and Delay** and specify which injectors you want to use and what volume you want to inject.
   • P-injector injects first (Pre-injector) and M-injector injects second.
   • You will have to experimentally determine what volumes you need. Generally, 50-100 µl of ATP and Luciferin solutions is sufficient for 25-50 µl of cell lysate.

d. Select **Wells to Read.**
   • You may choose to read only certain wells in the microplate, or to read the entire plate.
   • Highlight the wells to be read. Wells must be contiguous. Only wells to be read will be visible in the data display.

e. Select **Injection Wells**.
   • For each injector, highlight the wells you want to inject. Note that the wells you select for the P-injector are shown with a red semi-circle, the M-injector wells are shown with blue. Wells can be selected in any order and de-selected if you make a mistake.

f. **Plate Type: YOU CAN ONLY READ A 96-WELL PLATE.**

• Settings are now complete. Click OK to accept the settings as shown.

4. Prime injectors:

• Place injector tubing into your tubes containing Luciferase Assay reagents. Luciferin mix should go to the P-injector and the ATP mix should go to the M-injector. If you only have one solution, use the P-injector.
• Select **Prime injectors…** from the **Control** menu. Accept the default value for 7 injections and click **OK**. You need ~2ml to prime injector tubes.

5. Read plate by choosing Read Plate from the **Control** menu OR click the **Read** button in the toolbar of the plate section. (You will not see any data until all your samples have been read.)

6. <u>WASH INJECTORS</u> – It is VERY important to keep the tubing clean for accurate injections!!!

• If you would like to recover your reagents from the tubing, select **Reverse Injectors…** from the **Control** menu and click **OK** . Otherwise, proceed to next step.

• Place <u>both</u> tubes into a 50ml Falcon tube containing ddH_2O. Select **Wash Injectors…** from the **Control** menu. Accept the default settings (30 injections) and click **OK** .

• Repeat this wash step with 70%EtOH (30 injections).

• Replace each injector tube into the empty Falcon tubes and perform a final "Wash" that will just dry the injector tubing.

• **<u>EMPTY THE WASTE CONTAINER IF IT IS FULL!!</u>**