

TD-20/20 Luminometer



Operating Manual

Dated: 02/20/2003

Version 2.5

Part Number 2020-998



TURNER DESIGNS

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TD-20/20 Luminometer Operating Manual

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**Model TD-20/20 Luminometer
Calibration Record Sheet**
(This information is specific to your TD-20/20 Luminometer)

TD-20/20 Luminometer Serial Number:	Date:
Factory ¹⁴ C Sealed LSC Standard Unit #:	Factory Standard Reading (RLU):
Initials:	Date:

Customer ¹⁴C Sealed LSC Standard Unit #*: _____

Customer Standard Reading*(RLU)

1. _____
2. _____
3. _____

Average Standard Reading:
Standard Deviation:
% CV**:

* Applies only if customer has purchased P/N 112-0220 Light Standard

** %CV= (Standard Deviation X 100)/Average

I. Introduction

A. Description

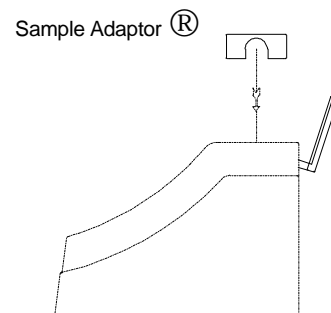
The TD-20/20 Luminometer is a sensitive, compact Luminometer designed for use in molecular and cellular biology using firefly luciferase as a genetic reporter; for measuring live biomass using the ATP assay; for use in immunological studies with chemiluminescence technologies; and for use in various biochemical applications. The sample chamber and adaptor are designed to accommodate a variety of sample holders. Sensitivity may be increased or decreased with ease using the keypad.

B. Inspection and Setup

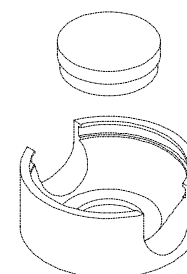
Upon receiving your Luminometer, please inspect it carefully and make sure all accessories are present (refer to the packing list shipped with the instrument).

To get started (refer to the Quick View Diagram, Section IIA):

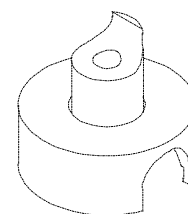
1. Consider the following factors for location:
 - a. Locate the instrument on a flat surface;
 - b. Since temperature can affect results, the work area should be free from hot or cold drafts and away from windows or heaters;
 - c. Many luminescent reagents are sensitive to light, particularly ultraviolet light. Therefore, when locating the instrument:
 1. Avoid sunlight;
 2. Avoid fluorescent lighting;
 3. Incandescent lighting is usually acceptable; reflected light is preferred to direct light.
2. Open the sample chamber lid located on the top of the unit. The lid will have a little resistance. Avoid leaving the lid open for extended periods of time.
3. Remove the sample adaptor by grasping and pulling up.
4. If using the range extender, See Section X for details.
5. Position the sample adaptor in the correct position for your needs. Refer to Section C for a detailed explanation of the TD-20/20 Sample Adaptor.
6. Reinsert the positioned sample adaptor by sliding it into the chamber. Insert the correct sample holder for your needs. Close the sample chamber lid. The lid has been designed to prevent sudden closure, so there will be a little resistance.
7. If the auto-injector system will be used, set it up at this time. See Section VI.
8. Plug in the unit. **Be sure to use only the Turner Designs power supply provided with your instrument.** It is critical to use the power supply provided by Turner Designs to meet EMI requirements.



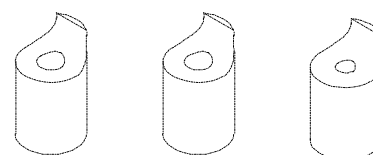
TD-20/20 Side View



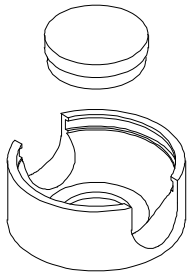
Sample Adaptor and 35 mm culture dish



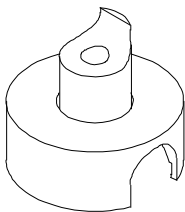
Sample Adaptor and Holder



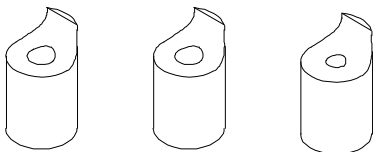
1.5 ml Microfuge Tube Holder, 8 mm Test Tube Holder, and 12 mm Test Tube Holder



Sample Adaptor and 35 mm culture dish



Sample Adaptor and Holder



1.5 ml Centrifuge Vial Holder, 8 mm Test Tube Holder, and 12 mm Test Tube Holder



9. Turn on the power switch (rear of unit) and allow the unit to warm up for the countdown period (300 seconds; 5 minutes).

C. Sample Adaptor and Holders

The TD-20/20 has been designed to hold a variety of sample containers. It will accommodate: 8 x 50 mm tubes; 12 x 50 mm tubes; 28 mm scintillation vials; 35 mm culture dishes; and 1.5 ml microfuge tubes.

Installing and Removing Sample Adaptor and Holders

The TD-20/20 Luminometer comes equipped with a sample adaptor that accommodates 35 mm culture dishes; when "flipped over", it will hold the 28 mm scintillation vials, or sample holders. Sample holders are available for 8 x 50 mm test tubes, 12 x 50 mm test tubes, and 1.5 ml microfuge tubes. These holders slide into the 28 mm opening of the sample adaptor.

To switch from 35 mm culture dishes to 28 mm vials:


1. Open the lid to the sample chamber.
2. Grasp the sample adaptor by the rim and pull straight up.
3. Remove any samples present.
4. "Flip" it over and slide it back into the chamber until it is fully seated.

To install the 8 x 50 mm, 12 x 50 mm, or 1.5 ml microfuge tube adaptors:

1. Open the lid to the sample chamber.
2. Make sure the sample adaptor is facing properly (see the drawing to the left).
3. Slide the appropriate holder inside the sample adaptor and push down until it is fully seated.

D. Definition of Symbols

 Direct Current. 12 volts D.C.
Gleichstrom

 Caution. Read instruction manual and refer to warning text.
Vorsicht. Lesen Sie die Anweisungen und die Warnung.

E. General Precautions

1. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.
Sollte das Gerät in einer Weise benutzt werden die nicht vom Hersteller spezifiziert ist, kann es möglich sein das die Wirkungskraft des Instruments auf diese Weise beschädigt wird.

Before Sensitivity Adjust or Reading Samples

2. Materials Needed

- Sufficient sample containers. Sizes: 8 x 50 mm tubes; 12 x 50 mm tubes, 28 mm scintillation vials; 35 mm culture dishes; 1.5 ml microfuge tubes.
- Assemble and prepare any reagents necessary for the assay to be run. Refer to any applications notes or procedures available.
- Samples to be read.

3. When Handling Samples

- Take care not to spill samples into the sample chamber. Wipe up any spills promptly. See Appendix 7, Section A, for details on maintenance.
- The cuvette **MUST BE DRY** on the outside when taking readings. Moisture on the outside will result in error.

4. Temperature Considerations

Most luminescent reactions will be measured at ambient temperature. As long as large temperature variations do not occur during a run, measuring the standard(s) and samples at the same temperature will be sufficient to ensure accuracy. ATP determination is an extreme example of this. Despite the fact that the reagent is an enzyme, temperature variation of several degrees will not result in significant error.

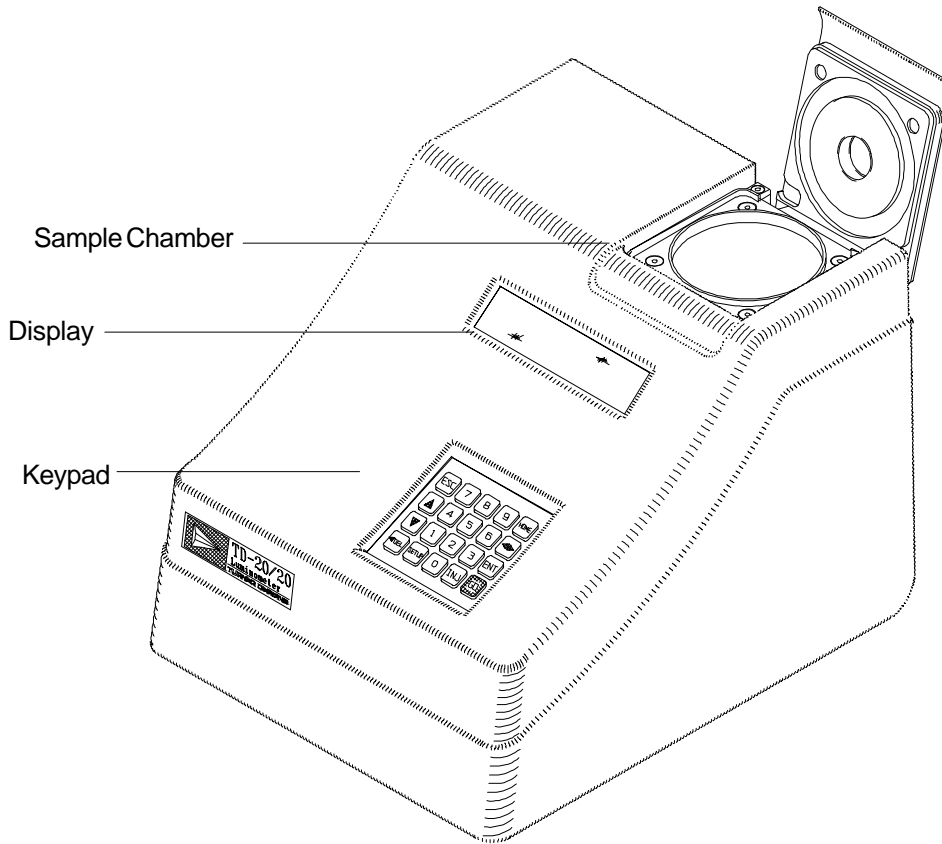
Additional temperature control will be desirable when:

- Laboratory temperature varies widely;
- Operation will occur at a temperature other than ambient.

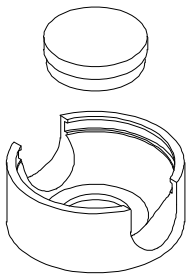
To provide additional temperature control, obtain a waterbath that meets necessary temperature requirements and sample and reagent capacity.

II. Hardware Overview

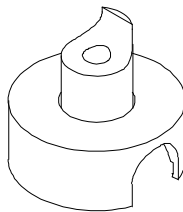
A. TD-20/20 Quick View Diagram



TD-20/20 Luminometer



**Sample Adaptor
and 35 mm culture dish**



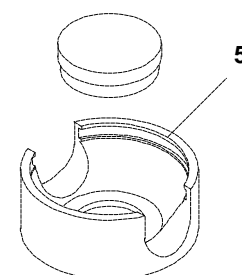
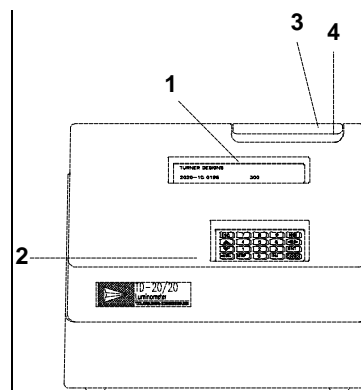
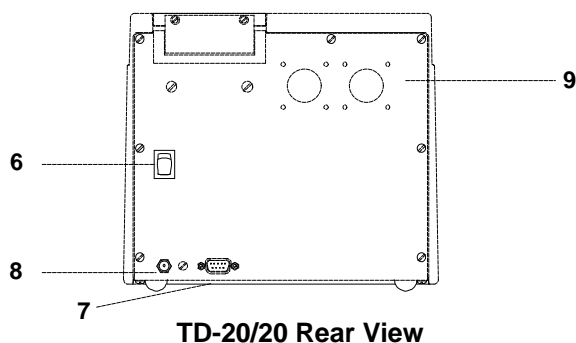
**Sample Adaptor
and Holder**



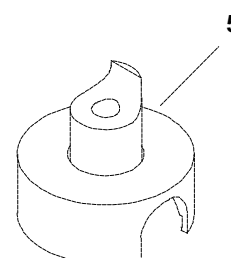
**1.5 ml Microfuge Tube Holder,
8 mm Test Tube Holder, and
12 mm Test Tube Holder**

B. TD-20/20 Features

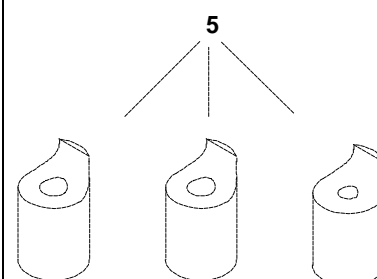
1. **Display** - Shows the screens and readout for samples. It is lit continuously when the unit is operating. Except during Sensitivity Adjust, the contrast of the display can be adjusted on any screen by pressing the up or down arrow keys.
2. **Keypad** - The keypad is used to enter new values and to move through the software screens.
3. **Lid** - The lid has been designed to ensure proper closure and to maintain a light seal. To prevent sudden closure, the lid is hinged and some resistance will be felt when closing it. The lid is designed to hold an auto-injector delivery system, which screws into the opening in the lid. If the TD-20/20 is not set up for auto-injection, the opening will be sealed with a black plug.
4. **Sample Chamber** - Samples are placed inside the chamber for reading. The chamber houses the sample adaptor, sample holder, range extender & LightSwitch™ module.
5. **Sample Adaptor and Holders** - The sample adaptor accommodates 35 mm culture dishes; when "flipped over" it holds 28 mm scintillation vials, and the sample holders. Sample holders, which slide inside the sample adaptor, are available for 8 x 50 mm round test tubes, 12 x 50 mm round test tubes, and 1.5 ml microfuge tubes.
6. **Power Switch** - ON/OFF switch.
7. **RS-232 Serial Port Connection** - RS-232 (DB9 female from the TD-20/20) serial port for connecting to a computer or printer.
8. **Power Plug Socket** - The power supply connects into the rear panel of the instrument.
9. **Auto-Injector** - The auto-injector is mounted inside the rear panel of the instrument and is activated from the TD-20/20 keypad.



**Sample Adaptor
and 35 mm culture dish**



Sample Adaptor and Holder



**1.5 ml Microfuge Tube
Holder, 8 mm Test Tube
Holder, and 12 mm Test Tube
Holder**

III. Instrument Parameter (Firmware)

A. Firmware Flowchart

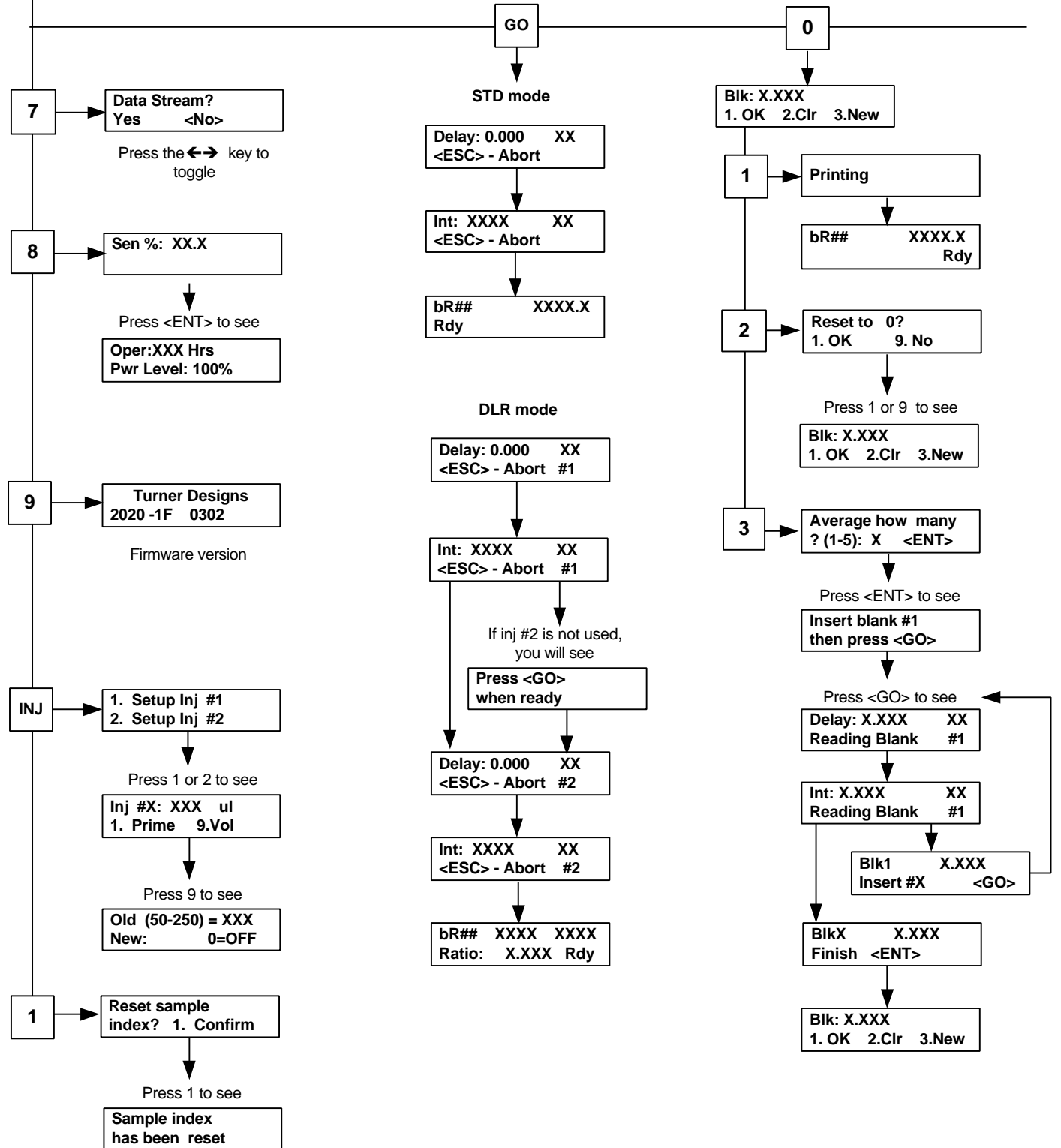
Turner Designs
2020 -1F 0302 300

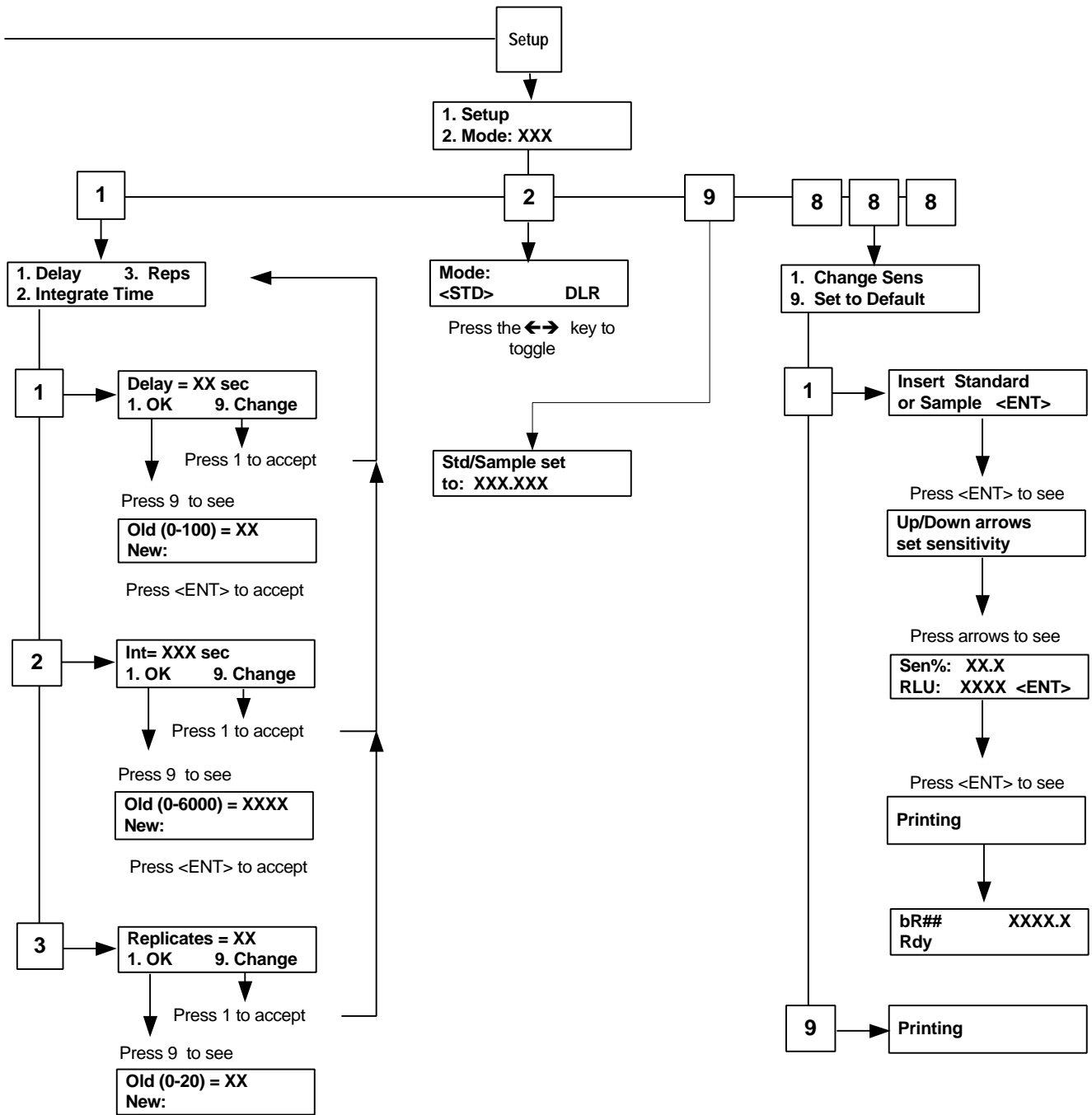
Warm up screen:
Countdown from 300

Home Screen:

b indicates blank subtraction activated
R# represents current replicate, if replicate is >1
XXXX is the reading of the last sample

bR## XXXX.X
Rdy





If a value is entered > stated range, you will see:

Invalid Input
<ENT> to reenter

Turner Designs 2020-1F 0302 300

Power-up Screen

bR# XXX.XXX Rdy (or) ALM
--

HOME Screen

1. Setup 2. Mode

Setup/Mode Screen

III. Instrument Parameters (Software)

Instrument parameters are set through the TD-20/20 Firmware interface. Screens are called up using the keypad. Use Sections III-X for explanations of the various parameters. For details about alarms and instrument diagnostics, see Appendix 5.

B. Power-up Screen

When the unit is first turned on, a screen appears showing the title, software version, and a 300-second (5-minute) countdown. Once the 300-second countdown expires, the HOME screen will appear. The countdown allows the instrument to warm up adequately before measurements begin. For optimal stability and accuracy, it is recommended that you allow the instrument to complete the countdown period.

If you wish to bypass the countdown period, press <HOME>, <ESC>, or <ENT>. From the HOME screen, press <9> to return to the Power-up screen.

C. HOME Screen

After the countdown period, the HOME screen is displayed. The HOME screen is where samples are read and data is sent to the printer or computer. The reading on the HOME screen will be static until <GO> is pressed to activate the integration sequence. The HOME screen displays sample readings to 4 significant figures (0.001 to 9999). To the left of the sample reading is the replicate number. A 'b' to the left of the replicate number indicates that a blank is stored in the instrument's memory and is being subtracted.

Press <HOME> to return to this screen from any screen (except during the Sensitivity Adjust sequence).

From the HOME screen, several functions can be accessed.

- Press <GO> to begin the "Integration" sequence (See Section III E for details). If an auto-injector is being used, this will trigger the auto-injector.
- Press <8> for Diagnostic Screens.
- Press <ESC> when an alarm is active (ALM) to view the alarm.
- Press <SETUP> to access the Setup/Mode Screen.
- Press <7> to turn on the data stream option.
- Press <0> to subtract blank.
- Press <1> to reset sample #.

D. Setup/Mode Screen

From the Setup screen, accessed from the HOME screen by pressing <SETUP>, the user can review or change the setup parameters. Pressing <SETUP> again from this screen sends the current Sensitivity Adjust information to a printer or computer.

E. Setup Screens

To access the SETUP screens, from the HOME screen press <SETUP>, then press <1> to access the Setup parameters. From the SETUP screen, press the appropriate number to access the parameter: <1> Delay; <2> Integrate Time; <3> Replicates. To change a value, press <9>, then enter the new value on the keypad and press <ENT>.

Integration Sequence. When a sample is read, the reading is integrated, or “summed” for a user-set Integrate period after a user-set Delay period.

1. **Delay Period.** The delay period is part of the Integration sequence. The Integration sequence is started from the HOME screen by pressing <GO>. The Delay period is the time the instrument waits before the onset of the Integrate period after <GO> is pressed. The delay allows time for the sample to stabilize, and for such factors as fading away of light-induced luminescence. The default Delay period is 3 seconds; the range is 0-100 seconds.
2. **Integrate (Int) Period.** The Integrate period is the time over which the instrument averages the reading of a sample. The Integration sequence is started from the HOME screen by pressing <GO>. After <GO> is pressed, the Delay period will run, followed by the Integrate period. If the TD-20/20 is connected to a printer or computer, the sample reading will printout automatically at the end of the Integration sequence. The default Integrate period is 15 seconds; the range is 4-6000 seconds.
3. **Replicates.** This screen allows the user to select the number of replicates (measurements of similar samples). If 1 is selected, the instrument will read and display the one sample only. If 2 or more replicates are selected, the instrument will read the first sample and display the measurement at the end of the integration sequence. The next replicate is then inserted and <GO> is pressed to start the reading. After all replicates are analyzed, the instrument will print or send to a computer the average of the replicates, the % variation of the measurements, and the standard deviation of the measurements. A replicate reading can be deleted and rerun by pressing the delete key after the sample is read. The default number of replicates is 1; the range is 1-20.

NOTE: The statistics cannot be viewed unless the TD-20/20 is connected to a printer or computer. See Appendix 1 and 2 for further details.

F. Mode Screen

To access the MODE screen, from the HOME screen press <SETUP>, then press <2> to access the MODE screen. Toggle between DLR and STD modes by pressing the left/right arrow key. If you are using Promega's dual luciferase reporter (DLR) assay or using the LightSwitch™ module for dual color assays, you may operate in the DLR mode. Otherwise, you should operate in the standard (STD) mode. For more information on Promega's DLR assay, contact Promega at 800/356-9526.

1. Delay	3. Reps
2. Integrate Time	

Setup Screens

Mode:	
<STD>	<DLR>

Mode Screen

IV. Subtracting the Reagent Background

In some cases, reagents have an intrinsic background luminescence that contributes to the luminescence readings of samples and standards. Subtracting the background from these readings results in greater accuracy in measurements. This procedure is called blank subtraction. The blank subtraction technique used here involves running one or more blanks, averaging the results to get a composite blank value, and subtracting this value from any subsequent sample measurements.

1. Press <0> from the HOME screen to start the procedure. The screen shown will display the current blank value(s) and show three choices
 - a) 1. OK – Choosing this option will send the current blank data to the printer and return the user to the HOME screen.
 - b) 2. CLR – Choosing this option will clear the current blank data from the luminometer's memory and reset it to 0.000.
 - c) 3. New – Choosing this option will allow the user to run up to 5 blank samples and average the data to form a new blank value.
2. If you elected to run a new blank, "Average how many? (1-5)" will be displayed on the screen. Enter the number of blanks you wish to measure. The average of the blank measurements will be used as the final blank number to be subtracted from the sample reading.
3. "Insert blank #1 then press <GO>" will be displayed. Press <GO> to run the first blank.
4. After the blank is complete, the blank value is displayed. The user is prompted to press <GO> to run the next blank. Remove the first blank sample and insert the second blank sample before pressing <GO>.
5. Repeat step 4 until all the blanks are run.
6. Normally, the user would skip this step and move on to step 7, but if you make an error running one of the blanks in step 5, press the <ESC> key to delete the most recent blank run, or to quit the blanking procedure without completing it. When pressing <ESC>, the user sees "Delete Last?" with three choices: 1.No, 2.Yes, 3.Quit
 - a) 1. No – The user selects this option if he does not want to delete any blanks that have been run. Choosing "No" returns the user to the previous screen.
 - b) 2. Yes – Selecting this option deletes the last blank run and returns the user to the screen where another blank can be run in its place.
 - c) 3. Quit – Selecting this option allows the user to quit and keep the blanks already run, or quit and ignore any blanks run.

7. Following the last blank, the message "Finish <ENT>" will be displayed on the bottom line of the screen. Press <ENT> to see the new average blank value.
8. The screen that appears is the same as in Step 1. Choose "1. OK" to print the blank value. The user is now returned to the HOME screen.
9. To alert the user that there is a blank value stored and being subtracted from the measurements, a "b" appears in the upper left corner of the HOME screen display.

Since there are two modes of analysis in the TD-20/20, DLR and Standard, there are two different blanks. The blank values saved in the DLR mode are not transferred to the Standard mode (or visa versa).

NOTE: Toggling between modes does NOT erase the stored blank values of either mode.

Data Stream
 <Yes> No

Data Stream Screen

**Section V.
 Data Stream Capabilities**

Several luminometric applications require the ability to monitor luminescence over time. The data stream feature allows this capability. When the data stream feature is on, the TD-20/20 outputs the real-time data continuously through the RS-232 port until actively stopped. This data may either be received by a printer or a computer.

General Information:

- Parameters in the Setup Screen (Delay, Integrate, and Replicates) are not active during Data Stream analysis
- Both or Either Injector can be used. The 1st injector will activate with pressing the [GO] button. The 2nd injection will start immediately after the 1st injection finishes.
- The Data output rate is fixed at 5 readings/second

Procedure:

1. From the "Mode" Screen, set the TD-20/20 to STD mode. Press the [HOME] key to return to the Home Screen.
2. Press [7] to access the Data Stream Screen.
3. Use the <-> key to toggle the parenthesis to select "yes".
4. Press the [HOME] key.
5. Set up the Auto Injectors, if they are to be used.
6. Press [GO]. Real Time Data will be viewable on the LCD Screen, refreshed 1/second. Data sent out to a printer or computer will be at the rate of 5 readings/ second.
7. Press [ESC] to stop the readings.

Data Output:

With Printer or Terminal Program

0.000 - Current Value of Subtracted blank
 - Empty

200.0
 184.8
 188.9
 185.3
 189.8
 186.4
 196.7
 aborted

Data Stream Data
 every 0.2 seconds

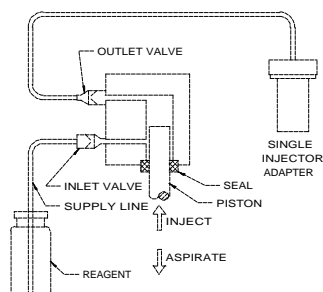
With Spreadsheet Interface Software

9.5069 - Time stamp in decimal format (9.5069 = 9:30:25AM)
0.000 - Current Subtracted Blank Value

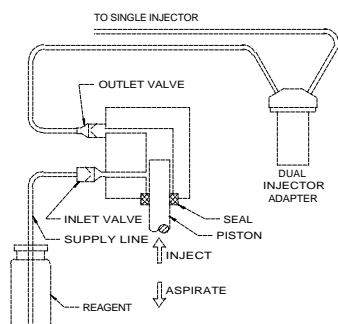
200.0
184.8
188.9
185.3
189.8
186.4
196.7
aborted

Data Stream Data
at 0.2s intervals

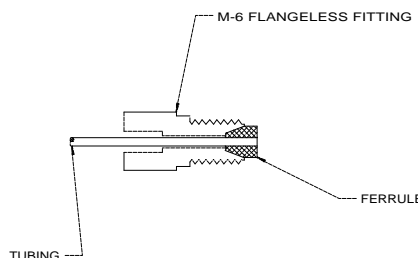
See Appendices 1-3 for further details on using printers or computer to capture/
report data.



Single Injector



Dual Injector



Fitting Connector



Injector Sample Holder

VI. Auto-Injector

A. Automatic Injector System - Mechanical

The TD-20/20 has been designed to operate with an automatic injector system. This factory-installed option is simple to operate and easy to maintain. Single or dual pump configurations are available.

It is important to understand the internal design of the injector pump because it allows the user to achieve optimum injector performance. The dual injector system consists of two sequential single injectors.

This system has been designed to use water-based reagents and solutions. It is critical that factory-supplied tubing be used. Please refer to the SPECIFICATIONS SECTION for fluid compatibility.

The injector pump operates by using a fluid displacing plunger to draw in and expel fluid. Flow is controlled by inlet and outlet check valves.

B. Installation (Configuring for Operation)

1. Using a coin, remove the black plug on the sample compartment lid.
2. Install the single or dual needle adapter into the sample compartment lid. The adapter should screw snugly into the lid to prevent any light leakage. Remove dust plugs from the injector pump head. Connect the injector tubing to the upper pump fitting(s).
3. Install the reagent bottle holder clip adjacent to the injector. Insert the reagent bottle into the holder clip.
4. Connect the supply line(s) to the inlet (lower pump fitting). Place the supply line into the reagent bottle and make certain it is at the bottle bottom.

NOTE: Exercise care in keeping contaminants such as lint, dirt, and dust from collecting on the supply line inlet. This can plug the injector valves and nozzle resulting in less than optimal performance.

5. At this point the supply and injector tubing is connected to the pumps, and supply tubing is inserted into the reagent fluid. The system is configured for normal operation. Proceed to the next section (Normal Operation).

C. Auto Injector Screens

The TD-20/20 injector(s) can be programmed to inject volumes between 50 and 250 μl in increments of 1 μl .

To program the injection volume:

1. From the HOME Screen, press <INJ>.
2. Select the specific injector by pressing <1> or <2>. The label on the back of the instrument will identify which injector is #1 and which is #2.
3. To program the volume, press <9>. Volumes may range between 50 μl to 250 μl . To turn off an injector, enter <0> as the volume. Press <ENT> then <ESC>.
4. Insert a tube or vial to catch any liquid pumped through during priming. Close lid.

NOTE: The acrylic window in the sample compartment is not compatible with certain solvents; including alcohols. Spills are to be avoided. Use a soft damp cloth to clean up any spills.

5. Press <1> to prime the injectors. This is to purge any air from the system. 4 priming cycles are normally adequate. Each priming cycle uses 250 μl .

NOTE: Check injector tip after priming for accumulations of droplets or bubbles. Remove droplets or bubbles with a soft dry tissue.

6. Injectors are ready for sample analysis.

D. Normal Operation

1. Insert a tube into the sample compartment.
2. Close lid.
3. From the Home Screen, press <GO> on the keypad. The Injection and Delay countdown will start immediately. If operating in STD mode, Injector #1 will inject first followed immediately by injector #2 if both have volumes programmed between 50-250 μl . If either has a volume of 0 μl , then only the active injector will inject reagent.

In DLR mode, the delay countdown and injection # 1 will start immediately. Once the integration is finished for the 1st reading, the second injection and delay countdown will begin. If injector #2 is turned off in DLR mode, the TD-20/20 will prompt the user to "Press <GO> when ready" so the user may hand pipet in a second reagent.

1. Setup Inj #1
2. Setup Inj #2

Inj#1	200 ul
1. Prime	9. Vol.

Old (50-250)=200
New: 0=Off

E. Daily Cleaning

Turner Designs recommends cleaning the injection system at the end of every day of use and when changing applications/reagents.

To clean:

1. Insert a tube or vial large enough to catch the liquid pumped through the injectors.
2. Remove supply tubing from the reagent bottles.
3. Using the priming cycle, purge all reagent from the injectors.
4. Insert supply tubing into DI water and prime the injectors 10 times to rinse the system.
5. Insert supply tubing into 70% ethanol solution and prime 6-10 times. Allow this solution to sit in the system for 1-16 hours for a thorough cleaning.
6. Purge the 70% ethanol solution and rinse systems 10 times with DI water.
7. Purge water.

F. Trouble-Shooting

This injector has been designed with materials and components offering superior reliability. Testing has shown that dirt and fluid contamination in the supply fluid has the greatest effect on pump performance. Contaminants can cause the injector tip to become obstructed or plugged. They also result in altering the performance of the check valves resulting in erroneous fluid metering. This requires a degree of care be used with regard to cleanliness. See Expanded Maintenance section below for detailed explanations of user maintenance.

<u>Symptom</u>	<u>Possible Cause</u>	<u>Corrective Action</u>
Air bubbles after priming	Loose tubing connections.	Check tubing fittings at inlet and outlet for tightness.
Injection weak and dripping	Obstruction in the tubing.	Remove affected injector supply and output tubing. Flush with clean water in opposite direction of flow or replace.
Injection weak and dripping after tubing replacement.	Obstruction in the pump check valves.	Remove valves flush injector and replace valves with new units.
Drip on injector needle tip, air in line.	The reagent fluid is depleted or the supply inlet tubing is above the reagent fluid level.	Add reagent. Place supply tubing inlet into the reagent.

G. Maintenance

As a general precaution, we recommend replacing the valves every 6 months and the tubing, nuts and ferrules every 12 months to ensure proper functioning of the injection systems. A replacement tubing and valve kit (P/N 2020-964) is available for this periodic maintenance.

See Section VI E for directions for Daily Cleaning.

Valve Replacement:

If the inlet and outlet flow valves become contaminated or obstructed, they may either leak or not pass fluid during the pump cycle. If the volume injected is inconsistent, it is likely that the valves need to be changed. You can avoid this condition by cleaning the injectors at the end of each day of use (See Section VI E). To replace the valves do the following:

1. Disconnect the sample input and sample output lines at the injector by unscrewing the end fittings.
2. Unscrew and remove the bolt that is at the center of the injector head.
NOTE: Be careful to not scratch the injector head.
3. Remove the circular injector head and place on a clean, dry, lint-free surface. Use a lint-free laboratory wipe.
4. There are two valves to replace, one on the injector head and one on the injector body mounted in the instrument.

NOTE: Remove valves and place in a separate area. Old valves are easily confused with new valves.

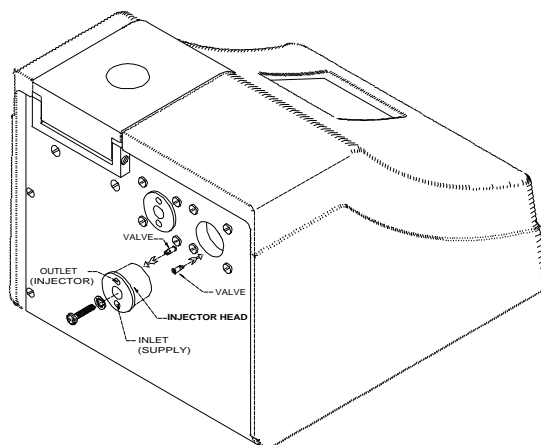
Use a pair of blunt-tipped tweezers to gently remove the small duck-bill valve from the injector head and replace it with a new valve. Gently remove the valve on the main body and replace with a new valve. Be careful not to damage the valves during installation or removal. Squeezing new valves open before installation can be helpful.

5. Replace the circular injector head by positioning the metal alignment pin on the injector head in the 9:00 o'clock position to match the pocket in the main body. Make sure the alignment pin on the injector head matches with the recession in the injector body. The injector head will lock in and not rotate when properly seated. If it rotates, gently push inward and turn injector head slowly until it is seated and can no longer rotate.
6. Screw in bolt at center of the injector head.
7. Reconnect the supply and injector lines by tightening the end fittings.
8. Prime the injector and check the output flow leaving the needle.

Note:

Before replacing the valves, observe if the existing valves are stuck closed. Remove the valves, pinch them open with your fingers, then reinstall. If the pump still performs poorly, replace the valves.

Valve Replacement



Tubing Replacement:

If the tubing becomes plugged or damaged, reagent either will not be dispensed or it will be dispensed at a fraction of the proper volume. It is best to replace both the inlet and outlet tubing at the same time unless it is certain which tubing is plugged. Supply tubing is a short section of PTFE tube. The output line is also PTFE but with the tip portion necked down for optimum fluid transition and low susceptibility to plugging. This pump is designed and calibrated for the factory-supplied tubing only. Using other types of needles and tubing will most likely result in unsatisfactory performance.

1. Disconnect the supply input and injector output tubing by unscrewing the Black M6 Nuts at the pump.
2. Remove the M6 nuts and ferrules on the lid adapter and carefully pull out the injector tube.
3. Unscrew and remove the lid adaptor.
4. With new injection tubing, slide 1 black nut and blue ferrule onto the narrow side end of the tubing. Position the nut and ferrule ~1.5 inches from the tip, to allow for ample tubing to protrude through the lid adapter by 1-2 mm. Take care not to crimp the tubing. Install tubing into lid adapter. See the fitting connector drawing by Section VI B for proper orientation of nut and ferrule. Install lid adapter back into the sample compartment lid.
5. Slide outer plastic jacket to be flush against the nut at the lid adapter. This prevents light from being conducted through the tubing into the sample adaptor.
6. Slide a nut and ferrule onto the other end of the injector tubing. This time have the ferrule flush with the edge of the tubing. Screw into the top opening of the injector head.
7. Slide a nut and ferrule onto the end of the supply tubing. Align the ferrule to be flush with the edge of the tubing. Screw into lower opening of the injector head.
8. If injectors were clogged before changing tubing, a thorough DI and ethanol cleaning may be necessary.

To Flush Air from the Injector:

An unprimed injector will dispense volumes less than desired due to air pockets in the reagent path. To flush this air, the injector should be primed. Follow the steps outlined in Section VI C Automatic Injector Screens - to prime the injector.

General Precautions:

1. Keep workspace environment clean. Avoid placing the tubing ends on table tops and dirty surfaces.
2. Flush the system with distilled water between reagents and after use.

3. Avoid storing system filled with reagents or salt solutions.
4. Prime injector 3-4 times before use.

H. Importance Notice to users of Promega's Dual Luciferase™ Reporter Assay

Proper Cleaning of Automated Injection Systems Used to Dispense Stop & Glo™ Reagent

Promega has recently determined that one of the luciferase quenching components in Stop & Glo™ Reagent has moderate affinity for plastic materials. This compound exhibits a reversible association with the interior surfaces of plastic tubing and pump bodies commonly used in the construction of auto-injector systems. Injector plumbing that has not been properly cleaned following contact with Stop & Glo™ Reagent will leach trace quantities of quench reagent into solutions subsequently passed through the injector system. In such cases, we find that even very small quantities of contaminating quench reagent cause significant inhibition of firefly luciferase reporter activity. Hence, proper cleaning of an injector system exposed to Stop & Glo™ Reagent is essential if the device is to be later used to perform firefly luciferase assays by auto-injecting either Luciferase Assay Reagent or Luciferase Assay Reagent II.

Washing the injectors with methanol as recommended in the *Dual-Luciferase™ Reporter Assay System Technical Manual* (#TM040, Section VII.C.5) has been found to be inadequate to completely remove residual Stop & Glo™ Reagent from injector assemblies. We recommend the following procedure for the cleaning of injector systems which have been used to dispense Stop & Glo™ Reagent.

Wash Protocol for the Injectors in the Turner Designs TD-20/20 Luminometer

The TD-20/20 Luminometer requires at least 5 priming cycles to achieve 100% displacement of the solution contained within the injector plumbing. Trace contamination of Stop & Glo™ Reagent may be removed from the TD-20/20 Luminometer injector system as follows:

1. Purge Stop & Glo™ Reagent from the injector by performing 10 priming cycles with deionized water (dH₂O).
2. Perform at least 6 priming cycles with a 70% ethanol cleaning solution (70% ethanol: 30% DI H₂O). Soak the injector plumbing in wash reagent **for at least 1 hour** (or up to 16 hours).
3. Perform at least 10 priming cycles with dH₂O to remove all traces of the 70% ethanol cleaning solution.

I. Specifications

Minimum injected volume	50 μ l
Maximum injected volume	250 μ l
Volume resolution	1 μ l
Precision	\pm 3%
Accuracy	\pm 5% for volumes \geq 100 μ l \pm 10% for volumes < 100 μ l
Inlet and outlet connection	M-6 x 1
Pump head retaining screw	#8-32 x .75
Tubing, Supply	.062 O.D. x .038 I.D. PTFE 12" Long
Tubing, Injection	.062 O.D. x .038 I.D. PTFE 12" Long .010 necked orifice.
Fluid compatibility	Water, Water-based reagents.
Fluid compatibility, occasional (50% solution max.). Must perform immediate distilled water flushing.	Alcohols such as Methanol, Ethanol.
Fluid compatibility, limited (2% solution max.). Must perform immediate distilled water flushing.	Alkali's, dilute ammonia, sodium hydroxide.

VII. Reading Samples

Standard (STD) Mode:

1. Fill a clean test tube, dish, or cuvette with sample and insert it into the sample adaptor in the sample chamber. Close the lid.
2. Press <GO> to initiate the Integrate sequence; if an auto-injector is being used, a sample will be injected into the sample holder. See Section VI for injector setup.

During the user-set Delay period, "Delay" will appear in the left corner of the HOME screen, the countdown in the right corner. Once the delay period is over, the background is sampled and the integration period starts.

During the user-set Integrate period, "INT" will appear in the left corner, the countdown in the right corner. Upon completion, the reading will automatically be sent to a computer or printer.

NOTE: The range for sample readings on the HOME screen is 0.001 to 9999.

3. Remove the sample.
4. Repeat steps 1 - 3 until all samples are read.

Dual Luciferase Reporter (DLR) Mode:

Reading samples in the DLR mode is similar to reading in standard mode with the following exceptions:

1. The sample is read twice (once after each reagent addition) and a ratio between the two measurements is calculated.
2. If an injector is present in the #2 position, the two measurements are performed automatically. If no injector is present, the software will pause for the injection of a second reagent. Press <GO> when ready to start the second measurement.
3. In DLR mode, the same Delay and Integration times are used for both readings.

NOTE: Readings >9999

If a sample gives a reading of >9999, it means the light from the sample was too bright for the instrument to accurately measure. No data will transfer to the computer or printer. The instrument expects the sample to be diluted and reanalyzed. This is particularly useful when analyzing replicate samples or DLR samples.

bR#	XXXX	XXXX
Ratio:	XXXX	Rdy

HOME Screen in the DLR Mode

Press <GO> when ready

Pause Screen

VIII. Reset the Sample Number Index

When using a printer or computer to collect data, a sample number is printed out with each sample reading. If the instrument is used for multiple experiments or used by multiple users, then resetting this sample number back to 1 is convenient for analysis.

To reset the sample number index:

1. From the Home screen, press [1] from the keypad.
2. The next screen will prompt the user to confirm this action by pressing [1] again.

Press the [ESC] key if you want to cancel the action.

Sample number index is now reset to 1.

IX. Sensitivity Adjust

A. Why Adjust Sensitivity?

The TD-20/20 is adjusted by the manufacturer to an optimal level of sensitivity using a light standard or a typical sample. In most cases, it is not necessary to adjust sensitivity. If, however, more or less sensitivity is required for a study, sensitivity may be adjusted electronically using the keypad.

B. Procedure

The Sensitivity Adjust procedure is a single point adjust in which a standard or typical sample is run in order to set the optimal range and sensitivity of the instrument. The Up (↑) and Down (↓) arrows are used to increase or decrease sensitivity. If the TD-20/20 is connected to a computer or printer, the Sensitivity Adjust information will printout automatically at the end of the procedure.

1. To adjust sensitivity, from the HOME screen, press <SETUP>, then <888> for Sensitivity Adjustment. Press <1> to change sensitivity. Press <9> to set sensitivity to the default.
2. Insert a cuvette or container containing a standard or typical sample into the Sample Compartment. Press <ENT>.
3. Use the Up (↑) or Down (↓) arrows to adjust the Sens factor (sensitivity factor) to the desired level. When the sensitivity is satisfactory, press <ENT>. Sens factor range: 0-100; Relative Luminescence Unit (RLU) range: 0-9999.
4. If >9999 appears, it means that sensitivity is too high to read samples on-scale. Reduce sensitivity until readings are on-scale. (See step 6 for an alternate method of reducing sensitivity using the range extender optical filter.)
5. The new sensitivity data is automatically sent to a printer or computer when adjustment is complete.
6. **Using the range extender filter to reduce sensitivity.** Sensitivity may be reduced by a factor of ~250 by installing a range extender filter (P/N 20-069). Before installing the filter, try to adjust sensitivity using the Sensitivity Adjust.

To install the range extender:

- a. Remove the Sample Adaptor by grasping the rim and pulling it out of the sample chamber.
- b. Place the filter directly over the photomultiplier window at the bottom of the chamber (See Section X for further details).
- c. Replace the Sample Adaptor.
- d. Adjust Sensitivity using steps 1 - 3.

1- Change Sens.
9- Set to Default

Note:

Although the possible range for the sensitivity factor (Sens Factor) is 0-100%, the practical maximum is about 60%. Except in special circumstances, very little gain is seen above this level.

C. To View or Print the Last Sensitivity Adjust

1. To view the most recent Sensitivity Adjust data set, press <SETUP> from the HOME screen, then <9>. The Sensitivity Adjust sample/standard value, the RLU value as set on the "Sens factor" screen, will be displayed. Press <HOME> to return to the HOME screen. The Sensitivity Adjust stored consists of the sample value only, expressed in raw luminescence units (RLU), as run during the most recent sensitivity adjust.
2. To print the most recent Sensitivity Adjust data set, from the HOME Screen, press <SETUP> to reach the Setup/Mode screen. Then press <SETUP> to send the current Sensitivity Adjust information to a printer or computer.

D. To Abort the Sensitivity Adjust

To abort the Sensitivity Adjust, press <ESC> at any time during the Sensitivity Adjust sequence. Press <1> to abort or <ESC> to resume. The previous Sensitivity Adjust will be maintained if the sequence is aborted.

X. Range Extender (Optional)

A. Purpose

The Range Extender is a specially designed optical filter for the TD-20/20 to reduce the light signal reaching the instrument's detector. This item is especially useful when the luminescence of your samples are routinely too strong/bright for detection with the TD-20/20. The range extender will reduce your sample's relative signal strength by a factor of ~250 giving you **two additional orders of magnitude** in the dynamic range of your application.

B. How to Use:

To Install

1. Lift the wire handle up, so you may easily hold the range extender while placing it into the TD-20/20.
2. Place the range extender over the detector window in the sample compartment. It should lie flat over the window.
3. Push the wire handle flat to the side, so it does not interfere with the sample placement and readings.

To Remove

1. Lift the wire handle up and lift it out of the instrument.

C. To determine the correction factor for the range extender:

You will want to calculate a correction factor for samples analyzed using the range extender so the values can be appropriately adjusted to correspond to the results without the range extender. To do this, we suggest using standards or previously analyzed samples whose relative fluorescence values ranged between 50-9,000 RLU's without the range extender in the TD-2020. *If all samples are analyzed with the range extender installed, the correction factor is not necessary.*

Steps to Calculate:

1. Take a reading of the standard or sample without the range extender installed in the sample compartment (L_b = luminescence before).
2. Insert the range extender.
3. Take readings of the standard or sample with the range extender installed (L_a = luminescence after).
4. Repeat steps 1-3 for each sample or standard.
5. Determine the relationship between each pair of values. This can be done with linear regression or with a simple percentage relationship (L_b/L_a). Average this percentage over all replicates. Multiply all future values with the range extender by this number to correlate with samples analyzed without the range extender.



Range Extender

APPENDIX 1 The Printout

A. Printout Capability with a Printer or Computer

To use this function with a printer

1. Connect the TD-20/20 to a serial printer via the RS-232 port on the back of the instrument. If you ordered a printer from Turner Designs, the appropriate serial cable is included with the printer.
2. Press <GO> from the HOME screen to initiate the Integration sequence. When the sequence is finished, the readout will be sent to the printer.

To use this function with a computer

1. Connect the TD-20/20 to your computer via the RS-232 port on the back of the instrument. Use the DB9 serial cable included with your instrument.
2. The TD-20/20 signal is in ASCII format so you can use various communications programs to import data to your computer. On your computer, run the communications program you have chosen. For a step-by-step procedure using Windows™ 95 HyperTerminal, see Appendix 2. For instructions on using the Spreadsheet Interface Software, see Appendix 3.
3. Press <GO> from the HOME screen to initiate the Integration sequence. When the sequence is finished, the readout will be sent to the computer with an index number.

B. Sample Reading with a Printer or Computer

At the end of a sample measurement, the TD-20/20 sends out a sample number index (1 - 999) and the reading of the sample in "RLU" (Relative Luminescence Units) in ASCII format.

The sample number index can be reset to 1 via

1. Using the Reset Sample index command in the firmware.
See Section VIII for details.
2. Rebooting the instrument
3. Changing the sensitivity
4. Changing the Blank Subtract Value
5. Changing the Mode (STD and/or DLR)

An example of the indexing feature is illustrated by the left column in the sample printout below where only 1 replicate is selected (for example only):

```
Turner Designs: TD-20/20 Luminometer
                Ver. 2020-1F 0302
Delay Time: (sec)      1
Integrate Time: (sec)  4
Number of Replicates:  10
Sensitivity Level %:   45.2
Standard Reading:      101.391
```

Injection Volume #1: (ul) 0
 Injection Volume #2: (ul) 0
 Blank: 0.027
 Mode: STD

Samples:

1. 1.346
 2. 2.345
 3. 3.456
 4. 4.758

When the user sets the instrument to run replicates, the printout will indicate the replicate with an "r" following the sample index number. When all replicates have been run, the TD-20/20 will calculate and printout the average of the replicate sample readings, the standard deviation (STD. DEV.), and the "%CV" for the samples (see the formula below). Sample printout with replicates (for example only):

Samples:

1. r 1 1.346
 r 2 1.446
 r 3 1.456
 Avg = 1.416
 %CV = 4.296
 Std. Dev = 0.061

2. r 1 4.758
 r 2 4.658
 r 3 4.858
 Avg = 4.758
 %CV = 2.102
 Std. Dev = 0.100

%CV (percent coefficient of variance) formula:

$$\%CV = \frac{\text{STD DEV (replicates)}}{\text{Average (replicates)}} \times 100$$

The %CV depends on what is being measured; a lower number is preferred.

Refer to Section III for definitions of Delay Time, Integrate Time, and Replicates. Refer to Section IX for an explanation of Sens factor and Std/ Sample setting.

See Section V for examples of the Data Stream print out.

APPENDIX 2

Data Collection

You can use various communications software programs to import data to your computer. The signal is sent from the TD-20/20 in ASCII format. You may use the Spreadsheet Interface Software for data collection directly to Excel (See Appendix 3). Complete instructions are included with the software. To use Window's '95 (or later) HyperTerminal program to import data from the TD-20/20:

1. Connect the 9-pin connector that extends from the TD-20/20 to the serial communications port of your computer (COM1 or COM2) using a 9-pin serial cable. You may need a 9-to-25 pin serial adaptor. Obtain this at your local computer store.
2. In Window's OS, locate the HyperTerminal program in the Accessories directory and double-click on the icon to open it.
3. Give the New Connection a name such as TD2020.
4. In the Phone Number window, choose Direct to COM 1 or Direct to COM 2 depending on which COM Port that the instrument is connected to. (This can be the hardest part of the procedure. Different computer manufacturers use different conventions.)
5. Set the Port Settings to the following parameters : Bits per second: 9600, Data Bits: 8, Parity: none, Stop Bits: 1, Flow Control: Xon/Xoff.
6. Save the Connection by choosing the File menu and selecting Save.
7. To store the data to a file, choose the Transfer menu and select Capture. Give the captured file a name.
8. Acquire your data.
9. Stop the Capture when you are done by selecting the Transfer menu, and then by selecting Capture/Stop.
10. Access your data using Excel, Word, or other programs by opening the file you saved in step 7.

APPENDIX 3

Spreadsheet Interface Instructions

A. Introduction

These instructions are designed to introduce you to the TD-20/20 Spreadsheet Interface Software. The software allows you to capture data directly into an MS Excel spreadsheet on your PC.

B. Materials Required

- TD-20/20 Luminometer
- Spreadsheet Interface Software CD
- RS-232 Cable & Serial Adaptor
- Operating Manual
- PC loaded with Windows '95 or higher (The computer should have at least one serial port available to connect to the luminometer)
- MS Excel 5.0 or higher

C. Software Installation Instructions

Insert the CD into the CDROM drive of your PC. Use the "Windows Explorer" program or "Run" command on your computer to run SETUP.EXE on the CD. This will initiate the installation program. When the installation is complete, an icon named "Spreadsheet Interface Software" will appear on your PC desktop and in the Programs menu.

D. Connecting the TD-20/20 to your Computer

Plug the 9-pin RS-232 cable included in the TD-20/20 accessory kit into the 9-pin connector on the back of the TD-20/20. Plug the other end of the cable into the 9-pin connector on your computer (lower right in picture). Some computers have the 9-pin connector occupied by a mouse. In this case, use a serial adaptor 25F/9M provided with the software. Plug the 9-pin RS-232 cable into the 9-pin side of the serial adaptor and plug the 25-pin side of the serial adaptor into the computer. The location of the 25-pin male plug on the computer is located next to the mouse connection and on the same PC board (lower left in picture). Do not plug into the 25-pin female connection on your PC, since this is usually the parallel port. (upper connector in picture).

E. Starting the Program

Click on the Spreadsheet Interface Software icon. The program will start and you should be able to see the software start-up screen displayed in Figure 1.

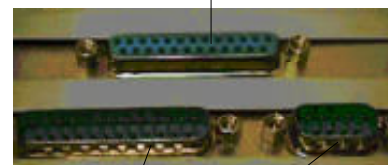
E. Selecting the COM port

The default port for instrument-computer connection is COM 1. If you connected the RS-232 cable to a different port, click on a button to the right of COM 1 (Figure 1). The COM port selection screen will appear (Figure 2).

On COM port selection screen, select the port corresponding to the RS-232 connection.

Backside of the Computer

Parallel Connector (25-pin Female). **DO NOT USE.**



25-pin Male Connector. Use if 9-pin male connector (right) is occupied by the mouse.

9-pin Male connector.

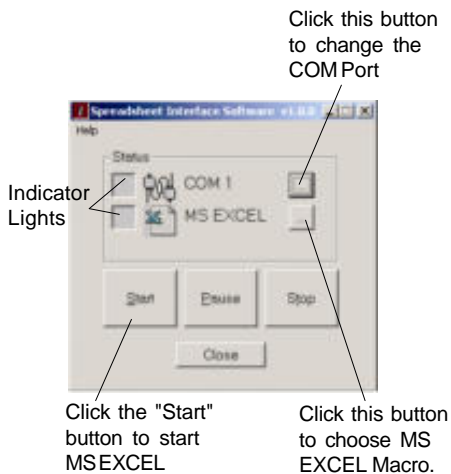


Figure 1: "Spreadsheet Interface Software" Start-up Screen.

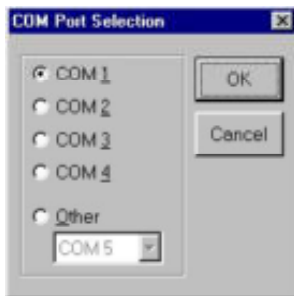


Figure 2

If the indicator light next to "COM 1" symbol is red (Figure 1), then the current COM port is not correct. Select another COM port and click on the "Start" button until the indicator light is green.

G. ACQUIRING DATA

If the correct COM port is selected, clicking on the "Start" button will successfully initiate the program and launch MS EXCEL. The indicator light next to MS Excel on the Start-up screen (see Figure 1) will be green. You are now ready to acquire data. Check the data output mode on the TD-20/20 by pressing <7> from the HOME screen on the instrument. If <NO> is selected, data will be exported after the sample run. If <YES> is selected, data will be exported at a rate of 5 times per second during the sample run. Press <GO> on the TD-20/20, and the data will appear in MS Excel according to the data output mode selected on the instrument.

If the indicator light next to MS is red, it means that the software cannot find the MS Excel directory. Click on the "Stop" button on the start-up screen. Launch MS Excel on your PC, then click on the "Start" button on the start-up screen.

H. Troubleshooting

Windows has a communications program called HyperTerminal included in every installation that can help you troubleshoot problems. When both indicator lights (next to COM1 and MS Excel) are green yet no data appear in MS Excel, follow steps A through G. This program is located in the "Accessories" directory in Windows '95.

1. Close Spreadsheet Interface Software
2. Open HyperTerminal.
3. Make a New Connection.
4. Give the New Connection a name.
5. Choose the COM Port that the instrument is connected to. (Choose the same COM port as selected in the Spreadsheet Interface software.)
6. Set the following parameters :
 - Baud Rate: 9600
 - Data Bits: 8
 - Parity: none
 - Stop Bits: 1
 - Flow Control: Xon/Xoff
7. Press <GO> on the TD-20/20 keypad.

If data appears here but did not appear when running the Spreadsheet Interface Software, please contact our technical support team at 408/749-0994 or Toll-Free 877/316-8049 (US & Canada).

If no data appears, then you most likely have a problem with

- Loose or damaged cable
- Wrong COM port selected
- COM port occupied with other software program

I. Pause, Stop, & Close

To pause the data collection without ending the program, click on the "Pause" button. Click on the same button, which will now say "Resume", to restart data collection.

To stop the data collection without shutting down the program, click on the "Stop" button. The program will now be inactive until you close the MS Excel spreadsheet and click on the "Start" button. Clicking on "Start" button without closing the MS Excel spreadsheet will result in a red light next to MSEXCEL.

To shut down the program, click on the "Close" button.

J. High Level Functions

Spreadsheet Interface software has the ability to call MS Excel macros.

The Spreadsheet Interface Software calls the MS Excel spreadsheet every time the software is launched. You can store customized MS EXCEL macros in this spreadsheet and have the program call these macros after data acquisition is complete. Examples of such macros include plotting data, performing statistical analysis of the data and others to successfully store a macro and have the Spreadsheet Interface Software run the macro. The following procedure should be followed:

1. Record the macro in Excel.
2. Save the macro in C:\Program Files\Spreadsheet Interface Software
3. Click on the button to the right of the MS Excel in the Spreadsheet Interface Software start-up screen. The "EXCEL configuration" screen will appear (Figure 3). Check the "Run Excel Macro" box, and enter the name of the macro.
4. Click on "Start" button and acquire data from the TD-20/20.
5. Click on "Stop" button.

The "Stop" command executes the MS EXCEL macro you recorded.

K. Data Format

The data acquired from the TD-20/20 Luminometer can be presented in two different formats in MS Excel.

Real Time Data:

The measurements for each sample appear in separate columns.

- | | |
|-----------|---|
| Row 1: | Time of day in decimal hours (e.g. 17.1572 is 5:09:25 pm) |
| Row 2: | Blank value subtracted from the measurement. |
| Row 3: | Empty. |
| Row 4+: | Real time data. Measurements are made every 0.2 seconds. The data shows both the delay and integration data. The total number of data points displayed is equal to the sum of the delay and integration times, in seconds, multiplied by 5. |
| Last Row: | "aborted" |

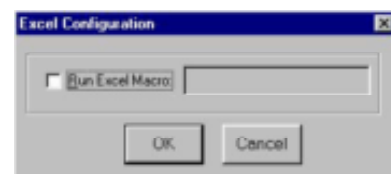


Figure 3. Excel Configuration Screen

The setup parameters can be transferred to the spreadsheet at any time by pressing the <SETUP> key twice from the luminometer's HOME screen. These parameters are displayed in columns G and J.

	A	B	C	D	E	F	G	H	I	J	K
1		15.83194	15.8375	15.84694	15.85389	15.85917		15.8622222	15.8625		
2		0	0	0	0		Turner Design: TD-20/20				
3							Luminometer				
4		247.6	275	296.1	305.1	194.3	Ver. 2020-1F 0302				
5		247.6	261.9	278.1	303.7	195.8	Delay Time: (sec)				3
6		242.5	283.6	294	297.2	195.8	Integrate Time: (sec)				10
7		244.1	295.2	290.7	300.5	195.2	Number of Replicates:				1
8		240.9	272.8	298.5	272.7	197.1	Sensitivity Level %:				46.4
9		244.1	273.7	300.6	297.2	205.1	Standard Reading				195.572
10		233.9	267.2	270.8	282.4	186.6	Injection Volume #1 (µl)				0
11		236	274.2	289.1	312.3	190.9	Injection Volume #2 (µl)				0
12		243.8	280.1	290.4	275.9	194.4	Average Blank:				0
13		252.6	259.9	297.3	282.1	186.5	Mode:				STD
14		245.6	279.3	300.1	287.4	190.7					
15		248.6	291.1	292.5	307.1	192.8					
16		238.9	277.6	280.4	288.8	196.3					
17		244.3	279.5	279.1	298.2	179.9					
18		243.5	269.9	282.9	298.8	181.8					
19		240	258.3	280.2	294.5	186.3					
20		245.1	279.3	289.3	305.8	201.9					
21		234.9	291.3	321.5	269.2	197.4					
22		aborted	aborted	aborted	aborted	aborted					

Integrated Data

Integrated data are displayed in rows. Each new measurement will be displayed below the previous one. This format is identical to that presented in Appendix 1, section B.

Setup Parameters can be transferred to the spreadsheet by pressing the <SETUP> button twice on the luminometer's HOME screen.

The sample number or "index" appears in column A. Integrated measurement appears in column B.

	A	B	C	D	E	F	G	H	I	J	K
1											
2											
3							Turner Design: TD-20/20				
4							Luminometer				
5							Ver. 2020-1F 0302				
6							Delay Time: (sec)				3
7							Integrate Time: (sec)				10
8							Number of Replicates:				1
9							Sensitivity Level %:				46.4
10							Standard Reading				195.572
11							Injection Volume #1 (µl)				0
12							Injection Volume #2 (µl)				0
13							Average Blank:				0
14							Mode:				STD
15											
16											
17		Samples									
18		1		189							
19		2		241.3							
20		3		278							
21		4		254.3							
22		5		294							

APPENDIX 4

Glossary

Accuracy	The degree to which a measured result approximates the true value of the quantity being measured. This value is usually expressed as a percent.
Analyte	The substance you wish to measure.
Sensitivity Adjust	Adjusting the sensitivity of the instrument to the standard and samples to be read.
Dark Current	A small amount of current flows in a photomultiplier tube even when the tube is operated in a completely dark state. This output is called dark current and the magnitude is greatly dependent upon the amount of voltage applied to the photomultiplier tube (i.e. the greater the Sensitivity Factor, the larger the dark current).
Detection Limit	May be used to refer to either the minimum or maximum concentration that can be read. Limits are specific to the luminescent material, the Sensitivity Adjust setting of the TD-20/20, sample volume, and distance of sample from light detector.
Drift	The change in measurement of the same sample over time.
Luminescence	The TD-20/20 Luminometer measures the concentration of various analytes in samples of interest via luminescence. A luminescent substance emits light under certain conditions, which can be detected by a photomultiplier tube.
Integration	A feature of the TD-20/20 which averages the sample reading over a user-set period of time after a user-set delay period. When <GO> is pressed from the HOME screen, after the user-set delay period, the signal will be summed for a user-set integrate time, and the reading displayed.
Noise	The amount of fluctuation in the instrument's measurement due to the instrument or detector itself.
RLU Luminescence	Refers to the "relative" luminescence of a substance being read, rather than the actual concentration. The range of "RLU Luminescence" units of the TD-20/20 is 0.001 - 9999.

Resolution

Resolution is related to sensitivity, but refers to the “detail” which can be read. When the instrument sensitivity level is high, lower concentrations can be read with ease (high resolution) but higher concentrations can not be read on-scale. When the sensitivity is low, higher concentrations can be read, but the detail is not as good at low concentrations (low resolution). Reading on the HOME screen: 0.001 to 9999 (4 digit limit).

**Sens factor
Sen %**

These terms are synonymous and are used to indicate the TD-20/20 Luminometer’s sensitivity on a scale of 1 to 100.

Sens % can be found on the diagnostic screen; it reflects the instrument’s current sensitivity setting as a percentage of the maximum possible sensitivity.

Sens factor is the term used in the software during Sensitivity Adjust. The Sensitivity Factor is directly proportional to the voltage on the photomultiplier tube. The higher the Sensitivity Factor, the greater the sensitivity (and typically, the greater the “noise”) of the readings.

Sensitivity

The basic operating level of the instrument. It is related to the detection limits and the resolution. The electronic sensitivity is set during Sensitivity Adjust using the keypad. Sensitivity can be adjusted mechanically using a neutral density optical filter. Sensitivity is also related to sample volume and distance from the light detector.

APPENDIX 5

Alarms, Diagnostics, and Troubleshooting

A. Alarm Screen

There are alarms built into the TD-20/20 to warn of an internal instrument malfunction or low power.

Table 1. Alarms

<u>Alarm</u>	<u>Delay</u>
High Voltage (HV Bad)	3 min.
Low Power (Low PWR)	1 min.

An alarm will be activated if the abnormal condition is in effect for a specified delay period. If an alarm is activated, "ALM" will blink on the HOME screen.

To see what alarm is active, from the home screen press <ESC>. For example, if the high voltage (HV) is outside the specified range you will see "HV Bad". If the HV alarm is triggered (HV Bad is seen when <ESC> is pressed from the HOME screen), contact Turner Designs.

If the low power alarm is triggered, check the power at the source and the power supply.

Refer to the Diagnostic Screens (next section) for definitions.

B. Diagnostic Screens

There are diagnostic screens which show the status of internal Luminometer functions. To access these screens, from the HOME screen, press <8>, then <ENT> to see the next screen in the sequence.

Definitions:

Sen %. Sen % is similar to Sens factor in the software. These terms indicate the Luminometer's sensitivity on a scale of 1 to 100. The Sen % is directly proportional to the voltage on the photomultiplier tube. The higher the number, the greater the photomultiplier tube voltage, the greater the sensitivity (and typically, the greater the "noise") of the readings. Sen % will display the current sample reading as a percentage of the RLU reading.

RLU. The "RLU" luminescence signal output from the instrument's light detector (the photomultiplier tube). This is the readout on the HOME screen. Range: 0 to 9999.

NOTE: To see the RLU luminescence for the standard/sample as set during the last Sensitivity Adjust, from the Setup/Mode menu, press <SETUP> to print or send to a computer. Or, from the Sensitivity Adjust & Setup menu, press <9> and it will appear on the display.

Operation Hour (Oper). Shows how many hours the instrument has been operating.

Power level. When at 100%, indicates power supply is functioning correctly.

B. Troubleshooting

<u>Symptom</u>	<u>Solution</u>	<u>Section To See</u>
Power switch ON, but no power	Make sure unit is plugged in securely; check power from the wall socket; Make sure unit's power supply is functioning. Make sure the green LED on the power supply is on.	Section II
Display reads >9999	Sample reading is too high for the instrument to read at the current sensitivity level. Use Sensitivity Adjust to reduce sensitivity. Or, install range extender filter.	Section IX + X
Low readings; not enough resolution	Sensitivity was not adjusted adequately during Sensitivity Adjust; readjust.	Section IV
Large Variation between replicates	If using injectors, changing the tubing and a thorough cleaning maybe required	Section VI
Air bubbles after priming	Check tubing fittings at inlet and outlet for tightness.	Section VI
Injection weak and dripping	Remove affected injector supply and output tubing. Flush with clean water in opposite direction of flow or replace.	Section VI
Injection weak and dripping after tubing replacement.	Remove valves flush injector and replace valves with new units.	Section VI
Drip on injector needle tip, air in line.	Add reagent. Place supply tubing inlet into the reagent.	Section VI

APPENDIX 6

Error Messages and Notes

During Sensitivity Adjust, the TD-20/20 will display an error message if incorrect values are entered or if the readings may result in an inadequate Sensitivity Adjust.

A. Invalid Input

This screen will appear when you enter a value that is outside the range for the parameter. For example, if you enter a number greater than 100 for the Delay, you will see "Invalid Input."

B. HOME Screen Displays >9999

If the HOME screen displays ">9999" (and the lid is closed), this means that the sample reading is too high for the instrument's light detector to read at the current level of sensitivity, i.e., sensitivity is set too high (RLU reading >9999). Reduce sensitivity using the Sensitivity Adjust procedure or dilute the sample.

When the screen display >9999 as a sample reading, no data will be transferred to the printer or computer. The instruments expect that sample to be adjusted and reanalyzed.

During Sensitivity Adjust, if >9999 is displayed, reduce sensitivity to obtain an on-scale reading. If sensitivity cannot be adequately reduced, install a range extender filter (P/N 20-069). See Section IX and X for further details.

APPENDIX 7

Maintenance, Warranty, and Service

A. Maintenance

Try to avoid spills into the sample chamber. If there is a spill:

1. Unplug the instrument.
2. Remove the test tube or dish and the sample adaptor from the instrument. Completely dry the sample adaptor.
3. Wipe up any moisture inside the sample chamber using a soft cloth. Do not use abrasives, solvents, or acids. Take care not to scratch, obscure, or damage the photomultiplier window at the bottom of the sample chamber.

Periodically wipe off the outside of the instrument with a damp cloth. Do not use solvents, acids, or abrasive cleaners to clean the TD-20/20.

B. Warranty

Turner Designs warrants the TD-20/20 Luminometer and accessories to be free from defects in materials and workmanship under normal use and service for a period of one year from the time of initial purchase, with the following restrictions:

1. The instrument and accessories must be installed, powered, and operated in compliance with the directions in this TD-20/20 Luminometer Operating Manual and directions accompanying the accessories.
2. Damage incurred in shipping is not covered.
3. Damage resulting from measurement of samples found to be incompatible with the materials used in the sample system is not covered.
4. Damage resulting from contact with corrosive materials or atmosphere is not covered.
5. Damage from sea water and other moderately corrosive materials that are not promptly removed from the instrument is not covered.
6. Damage caused by modification of the instrument by the customer is not covered.
7. Failure of limited life parts, such as injector valves & tubing, is not covered.

C. Obtaining Service

Warranty Service

To obtain service during the warranty period, the owner shall take the following steps:

1. Write or call the Turner Designs service department and describe as precisely as possible the nature of the problem.
2. Carry out minor adjustments or tests as suggested by the Service Department.
3. If proper performance is not obtained, ship the instrument, prepaid, to Turner Designs, with a statement of shipping charges. The instrument will be repaired and returned free of charge, along with a check to cover shipping charges, for all customers in the contiguous continental United States.

For customers outside of the contiguous continental United States, and who purchased our equipment from one of our authorized distributors, contact the distributor. If you purchased direct, contact us. We will repair the instrument at no charge, but we will not pay for shipment, documentation, etc. These charges will be billed at cost.

NOTE! Under no conditions should the instrument or accessories be returned without notice. Prior correspondence is needed:

- a. To ensure that the problem is not a trivial one, easily handled in your laboratory, with consequent savings to everyone.
- b. To specifically determine the nature of the problem, so that repair can be rapid, with particular attention paid to the defect you noted.

Out-of-Warranty Service

Proceed exactly as for Warranty Service, above. If our service department can assist you by phone or correspondence, we will be glad to, at no charge.

Repair service will be billed on a basis of time and materials. A complete statement of time spent and materials used will be supplied. Shipment to Turner Designs should be prepaid. Your bill will include return shipment freight charges.

Address for Shipment:

Turner Designs
845 W. Maude Ave.
Sunnyvale, CA 94085

APPENDIX 8

Specifications and Features

Sample Adaptors:	Standard sample adaptor holds 35 mm culture dishes or 28 mm scintillation vials; insert holders for 8 x 50 mm test tubes, 12 x 50 mm test tubes, or 1.5 ml microfuge tubes.
Sensitivity:	0.1 femtogram of luciferase.
Range:	5+ decades. 7+ decades with range extender.
Power:	External power supply, 100-240 VAC, $\pm 10\%$ of the nominal voltage. Max. 36 watts.
Dimensions:	23.5 cm W x 28 cm D x 21 cm H (9.25" W x 11" D x 8.25" H).
Weight:	3.6 Kg (7.9 lbs).
Operating Temperature:	5 - 40°C; 41 - 104°F.
Display:	16 x 2 character LCD.
Keypad:	4 x 5 keys.
Data Output:	100% ASCII format through a 9-pin RS-232 serial cable at 9600 baud.
Software:	Menu-driven microprocessor-controlled.
Detector:	Factory-installed photomultiplier tube. Peak Wavelength: 420 nm. Standard: 300 - 650 nm.
Printer:	Optional Seiko™ Printer (16.0 cm W x 17.0 cm D x 6.7 cm H).
Material:	UL-approved polyurethane. Sheet metal back and base.
Sensitivity Adjust:	Sensitivity can be adjusted electronically using the keypad in order to read more or less concentrated samples.
Integration:	User-set Delay period: 0 to 100 seconds; User-set Integrate time: 4 to 6000 seconds.
Injectors:	See specifications in Section VI.
Warranty:	One-year warranty.
Approvals:	TUV and CE.
Environmental Conditions:	Indoor use only. Altitude specification 0-2000m. Transient over voltages according to INSTALLATION CATEGORY II. Pollution degree 1 and 2 in accordance with IEC664.
Maximum Relative Humidity:	75% for temperatures up to 31°C, decreasing linearly to 50% relative humidity at 40°C.

ANHANG 9

Spezifikationen und Features

Probenhalter:	Der standard Probenhalter hält 35 mm Näpfchen oder 28 mm Küvetten; Halter für 8 x 50 mm Reagenzglas, 12 x 50 mm Reagenzglas, oder 1,5 ml microfuge Gläser.
Empfindlichkeit:	0,1 femtogram Luciferase.
Massbereich:	5+ Dekaden. 7+ Dekaden mit erweitertem Messbereich.
Strom:	Netzanschluß 100-240 VAC, max. 36 Watt ($\pm 10\%$ of the nominal voltage) $\pm 10\%$ der nominalen Stromspannung.
Abmessungen:	23,5 cm B x 28 cm T x 21 cm H.
Gewicht:	3,6 kg.
Arbeits-Temperatur:	5 - 40°C.
Display:	16 x 2 Stellen LCD.
Tastatur:	4 x 5 Tasten.
Datenausgabe:	100% ASCII-Format durch ein serielles 9-poliges RS232 Kabel bei 9600 Baud.
Software:	Menügesteuert, Microprozessor-kontrolliert.
Detecktor:	Eingebaute Photomultiplier Röhre. Spitzen Wellenlänge 420 nm. Standard 300-650 nm.
Drucker:	Optional Star™ Drucker (16,0 cm B x 17,0 cm T x 6,7 cm H).
Materiel:	UL-getestetes Polyurethan. Grundplatte und Rückseite aus Metall.
Empfindlichkeit-seininstellung:	Die Empfindlichkeit kann auf elektronische Weise eingestellt werden unter Benützung der Tastatur um mehr oder weniger konzentrierte proben zu erfassen.
Integration:	Vom Benutzer eingestellte Perioden 0 bis 100 Sekunden. Vom Benutzer eingestellte Integrationsperioden 4 bis 6000 Sekunden.
Injectors:	Benutzen Sie die Spezifikationen im Section VI.
Garantiezeit:	1 Jahr.
Geprüft:	von CE, TÜV und UL.
Umweltsbedingungen:	Kann nicht im Freien benutzt werden. Höhenspezifikation: 0-2000 Meter. Überspannungsspitzen laut Installationskategorie II. Verschmutzungsbereich 1 und 2 laut IEC664.

**Maximale
relative**

Luftfeuchtigkeit: 75% bis 30°C, linear abnehmend bis 50% bei 40°C.