

DIVISION OF DEVELOPMENTAL BIOLOGY & TOXICOLOGY

# **Working Manual Number: 01**

## **Instrument: Distillation**

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## **DISTILLATION**

### Switch ON

Step 1: Plug in the wire labelled with “Dist. Pack” to the switch board.

Step 2: Attach the water pipes connected to the condenser. Connect Pipe A to the tap (it will allow filling of tap water in the condenser) and Pipe B to the outlet for circulation of water. Then turn ON the tap and allow water to fill the outer tube of the condenser.

Step 3: Fill the distillation flask with RO water such that the inlet remains submerged in water. Keep adding RO water (to make distilled water)/ Distilled water (to make Double distilled water) to the distillation as and when the water level drops.

Step 4: Place the bottle for the collection of distilled water with the funnel below the outlet tube for condensed water.

Step 5: Turn on the switch and allow the coil to heat up (coil shall turn orange/red upon heating). As the coil will heat up water will start boiling, followed by the evaporation and condensation.

### SWITCH OFF

Step 1: Turn OFF the main switch of the distillation pack.

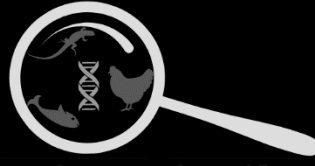
Step 2: Remove the funnel from the collection bottle, close the cork and keep the bottle at the designated storage location.

Step 3: Allow the distillation coil and condenser to cool for 30-60 minutes.

Step 4: Put of the tap of running water and discard the water from the condensation chamber.

### CLEANING

Disassemble and clean the distillation unit using diluted HCl to remove the condensed salts. Then wash the apparatus thoroughly and run the distillation to ensure proper cleaning of the unit. Discard the initial 100 ml condensed water to make sure the pH of distilled water is not disturbed due to HCl contamination.



DIVISION OF DEVELOPMENTAL BIOLOGY & TOXICOLOGY

# **Working Manual Number: 02**

## **Instrument: Hot Air Oven**

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## **HOT AIR OVEN**

### Switch ON

Step 1: Connect the main power cord labelled Oven 1 to the switch board.

Step 2: Place fresh, dry blotting papers on racks in the hot air oven.

Step 3: Turn ON the power switch, followed by the mains switch (on the oven). Keep the fan off.

Step 4: The temperature is pre-set to 58°C (most commonly used in lab). For change of temperature adjust to the required value using the panel on the instrument.

### Switch OFF

Step 1: Turn OFF the mains switch on the instrument.

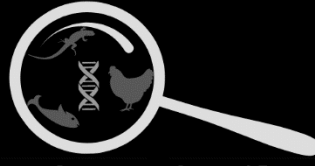
Step 2: Turn OFF the power switch on the main supply.

### CLEANING

Ensure timely replacement of the blotting papers.

(PS: DO NOT leave the hot air oven ON overnight)

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# **Working Manual Number: 03**

## **Instrument: Benchtop Cooling Centrifuge**

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## **CENTRIFUGE**

### **Switch ON**

Step 1: Plug in the wire labelled with “Sorvall” to the switch board. Then turn ON the switch. Next turn ON the stabilizer (in the cupboard below) (switch is on when upwards check the directions arrow). Wait for the stabilizer to make a start sound.

Step 2: Remove the cover of the instrument. Select and change the rotor to be used for the spin (big rotor for 50ml falcons or with holders for 15ml falcons/ small rotor for 1.5ml or 2ml MCTs).

Step 3: Turn ON the Mains switch on the Centrifuge (black switch on the right hand top corner in Thermo Scientific Sorvall 8T Centrifuge/ blue switch on the front panel for Sorval Super T21 Centrifuge).

Step 4: Adjust the RPM/g values at which the rotor is supposed to be set, set the time duration for the spin and the temperature.

Step 5: Press the OPEN button on the panel, open the rotor (by rotating clockwise/anticlockwise) and place the tubes to be centrifuged in a balanced manner inside the rotor then close the rotor (by rotating clockwise/anticlockwise). Check for the set values of speed, time and temperature and close the lid of the centrifuge with a soft push such that it makes a soft locking sound. Then press “Start”.

(PS: Avoid using the “Stop” command for the instrument as far as possible.)

### **SWITCH OFF**

Step 1: Once the spin is over and the centrifuge makes the beeping sound, press the OPEN button on the panel, open the rotor (by rotating clockwise/anticlockwise) and gradually remove the tubes which have been centrifuged then close the rotor (by rotating clockwise/anticlockwise).

Step 2: If further more spins are supposed to be done close the rotor and centrifuge lid leaving the instrument ON.

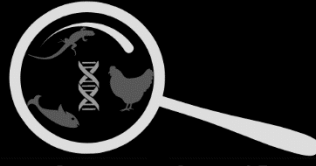
Step 3: If no further spins are supposed to be made, remove the rotor from the centrifuge and keep the lid open.

Step 4: Put OFF the mains switch (black switch on the right hand top corner in Thermo Scientific Sorvall 8T Centrifuge/ blue switch on the front panel for Sorval Super T21 Centrifuge). Turn OFF the stabilizer followed by the switch with the plug labelled “Sorvall”.

### **CLEANING**

Remove the rotor and clean the holders with Iso-P using tissues and ear buds after each spin. Wipe off the interiors of the centrifuge using a clean dry tissue to clean the condensed water.

Regularly oil the liners of the rotor to avoid sounds while spinning.



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# **Working Manual Number: 04**

## **Instrument: Weighing Balance**

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## **WEIGHING BALANCE**

### Switch ON

Step 1: Connect the main power cord to the switch board and turn on the switch and remove the cloth cover of the instrument.

Step 2: Turn ON the power switch on the instrument and wait for the display.

Step 3: Check for the placement of the bubble indicating a balanced state of the instrument. The bubble should be exactly in the center of the ring boundary.

Step 4: Use a weighing pan or paper boats (for non-hygroscopic chemicals)/ aluminum boats (for hygroscopic chemicals).

Step 5: Open the door of the weighing balance and place the weighing pan/boat on the weighing platform. Press the "TARE" button on the panel on the instrument to bring the basal weight to zero.

Step 6: Take the scalpel/spoon according to the amount of chemical to be weighed and clean it with tissue and Iso-P then proceed to weigh the chemical.

### Switch OFF

Step 1: Clean the scalpel/spoon with tissue and Iso-P.

Step 2: Clean the weighing platform using dry tissue, in case of sticky chemicals, use a wet tissue and immediately clean using a dry tissue to avoid rusting.

Step 3: Close the door of the weighing balance.

Step 4: Turn OFF the power switch on the instrument.

Step 5: Turn OFF the switch on the Main switch board.

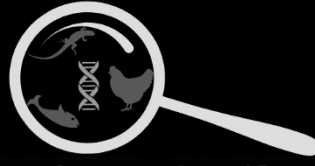
Step 6: Clean the work area with Iso-P and cover the instrument again with the cloth cover.

### CLEANING

Clean the instrument using dry tissues at regular intervals to avoid rusting and maintaining the instrument's efficiency.

(PS: Make sure the placement of the bubble is in the center to minimize the errors)





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# **Working Manual Number: 05**

## **Instrument: Weighing Balance**

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## **WEIGHING BALANCE (Mol Bio Room)**

### Switch ON

Step 1: Connect the main power cord to the switch board and turn on the switch and remove the cloth cover of the instrument.

Step 2: Turn ON the power switch on the instrument and wait for the display.

Step 3: Check for the placement of the bubble indicating a balanced state of the instrument. The bubble should be exactly in the center of the ring boundary.

Step 4: Use a weighing pan or paper boats (for non-hygroscopic chemicals)/ aluminum boats (for hygroscopic chemicals).

Step 5: Open the door of the weighing balance and place the weighing pan/boat on the weighing platform. Press the "TARE" button on the panel on the instrument to bring the basal weight to zero.

Step 6: Take the scalpel/spoon according to the amount of chemical to be weighed and clean it with tissue and Iso-P then proceed to weigh the chemical.

### Switch OFF

Step 1: Clean the scalpel/spoon with tissue and Iso-P.

Step 2: Clean the weighing platform using dry tissue, in case of sticky chemicals, use a wet tissue and immediately clean using a dry tissue to avoid rusting.

Step 3: Close the door of the weighing balance.

Step 4: Turn OFF the power switch on the instrument.

Step 5: Turn OFF the switch on the Main switch board.

Step 6: Clean the work area with Iso-P and cover the instrument again with the cloth cover.

### CLEANING

Clean the instrument using dry tissues at regular intervals to avoid rusting and maintaining the instrument's efficiency.

(PS: Make sure the placement of the bubble is in the center to minimize the errors)



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**Working Manual Number: 06**

**Instrument: Mini Spin**

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## **MINI SPIN**

### Switch ON

Step 1: Connect the main power cord to the switch board and turn ON the switch.

Step 2: Turn ON the power switch on the instrument.

Step 3: Arrange the MCTs to be spun in a balanced manner inside the instrument.

Step 4: Close the lid of the instrument and adjust the speed and time of the instrument.

### Switch OFF

Step 1: Open the lid and gradually remove the tubes.

Step 2: Turn OFF the power switch on the instrument.

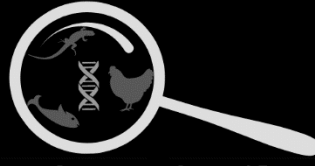
Step 3: Turn OFF the switch on the main switch board and remove the plug.

### CLEANING

Ensure timely of the tube holders using tissue and Iso-P.

[PS: Do not misplace the tube holders (white coloured) of the minispin.]

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# **Working Manual Number: 07**

## **Instrument: GeNei: Gel Imager**

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## **GeNei- GEL IMAGER**

### Switch ON

Step 1: Connect the main power cord to the switch board and turn ON the switch.

Step 2: Turn ON the power switch of the instrument and remove the cloth and plastic cover from the adjustment panel.

Step 3: Open the door of the Gel Imager and place the gel on the center of the base.

Step 4: Close the door of the instrument and turn On the UV light source. Adjust the focus and zoom using the fine adjustments (on top of the instrument). Adjust the intensity and exposure time of the UV light as per the requirement.

Step 5: Once the focus and exposure is adjusted, for obtaining the image of the gel, click the “capture” button on the instrument’s panel and click save. Also note down the auto-generated serial number of the image.

### Switch OFF

Step 1: Turn OFF the UV light and bring the intensity knob to medium intensity.

Step 2: Clean the UV illuminator base with a dry tissue followed by a tissue wettened with Iso-P. Close the door once the base has dried.

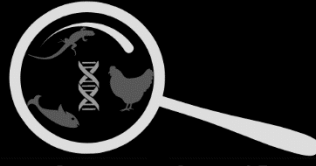
Step 3: Turn OFF the power switch on the instrument.

Step 4: Cover the fine adjustment levers with cloth and the plastic cover.

### CLEANING

Clean the UV illuminator base with dry tissue to clean the remanent buffer, followed by cleaning with tissue wettened with Iso-P.

(PS: DO NOT open the instrument without turning OFF the UV light source.)



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# **Working Manual Number: 08**

## **Instrument: E-Gel Imager**

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## **E-Gel IMAGER**

### Switch ON

Step 1: Connect the main power cord labelled UV light base to the switch board and the camera cord to its slot and the imaging device (laptop/ desktop). Then turn ON the switch.

Step 2: Turn ON the Power button on the instrument. Then open the E-Gel Imager software on the imaging device (laptop/ desktop) and select the UV-light base.

Step 3: Lift the cover the instrument and place the gel on the UV illuminator base. Then turn on the UV light button on the base of the instrument.

Step 4: Adjust the focus, sharpness, exposure and brightness according to the requirement then click on save to capture the image.

### Switch OFF

Step 1: Turn OFF the UV light and bring the intensity knob to medium intensity.

Step 2: Clean the UV illuminator base with a dry tissue followed by a tissue wettened with Iso-P. Close the door once the base has dried.

Step 3: Turn OFF the power switch on the instrument. And remove the cords.

### CLEANING

Clean the UV illuminator base with dry tissue to clean the remanent buffer, followed by cleaning with tissue wettened with Iso-P.

(PS: DO NOT open the instrument without turning OFF the UV light source.)





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# **Working Manual Number: 09**

## **Instrument: Laminar Air FLOW**

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## **LAMINAR AIR FLOW**

### Switch ON

Step 1: Turn ON the UV light for 5 minutes to create a sterile environment once daily.

Step 2: Turn ON the white light and air flow.

Step 3: Open the hood of the LAF and clean the floor using Iso-P and tissue. Then place the discard beaker and other required equipment on the LAF floor.

Step 4: Turn ON the burner whenever needed.

### Switch OFF

Step 1: Turn OFF the burner (if turned on), remove the discard beaker and close the hood.

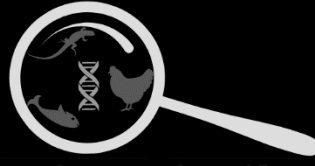
Step 2: Turn OFF the white light and air flow.

### CLEANING

Ensure cleaning the floor with tissue and Iso-P before and after use of the LAF.

(PS: DO NOT open the hood with UV light on. Remove the discard beaker without fail.)

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# **Working Manual Number: 10**

## **Instrument: Biorad i-mark Elisa Plate Reader**

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## **BIORAD iMARK ELISA PLATE READER**

### Switch ON

Step 1: Plug in the Biorad imark Elisa plate reader. Then press the ON button (on small screen the initializing will be visible for 1-2min).

Step 2: Once initializing done then insert the password and press 'enter'. Wait for the home screen to appear (Date and Time, Current filter, Shaking mode).

Step 3: Set the Blank -->Press edit, Mapping, Manual mapping, set the blank using blank button (no.2)/sample button (no.1), press Enter (the you will be redirected to home screen).

Step 4: Set various filters: Press edit, Cutoff, Filters (1: 415nm, 2: 450nm, 3: 490nm, 4: 655nm, 5: 595nm, 6: 750nm, 7: 570nm, 8: 560nm) according to the requirement set the filter, press 'Enter'. You will be redirected to home screen where the filter chosen can be seen)

Step 5: Press 'Open' to obtain the plate holder. Insert the plate in the instrument. The press 'Close'.

Step 6: Press 'Start' to obtain the results. The machine will start reading the plate and subsequently the report will be generated.

### Switch OFF

Step 1: Once the reading has been done remove the plate from machine.

Step 2: Long press the 'Power' button it will ask you 'Power Off?' Select 'Yes' from the options blinking on the screen using the enter key.

### CLEANING

Ensure cleaning the plate holder in case of spillage.

(PS: DO NOT leave the machine ON after the work is completed.)



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# **Working Manual Number: 11**

## **Instrument: Rocker**

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## **ROCKER**

### Switch ON

Step 1: Connect the main power cord to the switch board.

Step 2: Place fresh, dry blotting papers/ white one sided paper on the rocker.

Step 3: Turn ON the power switch, followed by the mains switch (on the rocker).

Step 4: Adjust the time duration for which the instrument is supposed to run using the arrow keys on the panel on the instrument. Press 'Enter' to start the rocker.

### Switch OFF

Step 1: Once the preset time duration is over the rocker will automatically stop. If you need to stop before the set time duration press 'Clear'.

Step 2: Once the run is over, turn OFF the mains switch on the instrument.

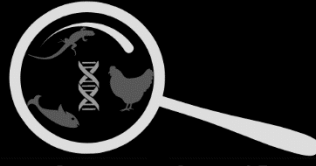
Step 3: Turn OFF the power switch on the main supply.

### CLEANING

Ensure timely replacement of the blotting/ one sided white papers.

(PS: Adjust a moderate speed for the rocker to maintain the instrument.)

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# **Working Manual Number: 12**

## **Instrument: Magnetic Stirrer**

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## **MAGNETIC STIRRER**

### Switch ON

Step 1: Connect the main power cord to the switch board.

Step 2: Turn ON the power switch, followed by the mains switch (on the rocker).

Step 3: Adjust the speed at which the instrument is supposed to run using the knob on the instrument.

Step 4: Place the reagent bottle on the stirrer after adding the magnet to it.

### Switch OFF

Step 1: Once the run is over, turn OFF the mains switch on the instrument.

Step 2: Turn OFF the power switch on the main supply.

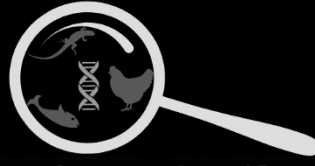
### CLEANING

Ensure cleaning of the instrument in case of spillage. Remove the magnet from the reagent bottle and clean it with distilled water and wipe of dry.

(PS: Adjust a moderate speed for the stirrer to maintain the instrument.)

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# **Working Manual Number: 13**

## **Instrument: BOD Incubator**

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## **BOD INCUBATOR**

### Switch ON

Step 1: Connect the main cord to the stabilizer. Switch on the Main switch for power supply.

Step 2: Switch ON the main key and wait till it displays the current temperature on the panel.

Step 3: For humidity, put a water-filled tray in the lowermost rack of the incubator.

Step 4: To check the humidity, an additional humidity measuring device has to be put inside the incubator.

Step 5: For changing the set temperature, press SET button given just below the display and by using the knob, adjust the temperature and press the SET button again.

### Switch OFF

Step 1: Turn OFF the mains switch on the instrument.

Step 2: Turn OFF the Main switch on the power cord on the switch board.

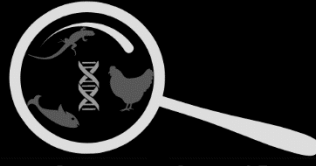
Step 3: Decant the trays placed inside the instrument for maintaining the humidity.

### CLEANING

Ensure timely cleaning of the instrument and water trays. Use blotting papers wherever necessary.

(PS: Before putting anything inside the incubator, please switch it on 20-25 min before.)

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# **Working Manual Number: 14**

## **Instrument: CO2 Incubator**

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## **CO2 INCUBATOR (FORMA ENVIRONMENTAL CHAMBER)**

### Switch ON

Step 1: Plug in the machine and switch on, now switch on the device by the main switch given on the front part of the incubator; when the switch is on, the green indicator will be on (just above the main switch).

Step 2: The above panel of the incubator has different parameters like CO2 levels, temperature and humidity. Each panel has two display numbers; the upper one shows current levels of CO2/temperature/humidity and the lower one shows set levels (required levels) of the parameters.

Step 3: When the incubator is switched on, its siren will start; it indicates some parameter is not achieved (generally CO2) when the machine has started. To stop the siren, press easy (EZ) of CO2 parameter.

Step 4: To change the set parameter values, use up and down arrow buttons.

Step 5: For changing the set temperature, press SET button given just below the display and by using the knob, adjust the temperature and press the SET button again.

### Switch OFF

Step 1: Turn OFF the mains switch on the instrument.

Step 2: Turn OFF the Main switch on the power cord on the switch board.

### CLEANING

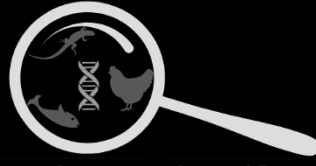
Before putting anything in the incubator, clean all the racks with methanol or isopropanol.

(PS: Before putting anything inside the incubator, please switch it on 20-25 min before.

For humidity, make sure the tank has enough water; if water is less, then refill distilled water and add chlorine in it to avoid any contamination through water.

For CO2 levels, make sure the CO2 cylinder is filled and opened with correct pressure.

When the incubator shows a heating indication when the required temperature has already been achieved, switch on the refrigeration. In the same way, when humidity exceeds the set values, switch on the dehumidifier, but as the set values are achieved, make sure the switches are put off.)



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# **Working Manual Number: 15**

## **Instrument: Vortex**

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## **VORTEX**

### Switch ON

Step 1: Connect the main power cord to the switch board and turn ON the switch.

Step 2: Turn ON the power switch on the instrument. Adjust the rotational speed using the knob on the instrument.

Step 3: Place the MCT/ falcon to be vortexed on the holder.

### Switch OFF

Step 1: Turn OFF the power switch on the instrument.

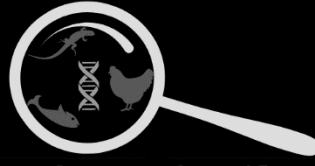
Step 2: Turn OFF the switch on the main switch board and remove the plug.

### CLEANING

Ensure timely of the tube holders using tissue and Iso-P.

(PS: DO NOT leave the instrument on after work.)

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# **Working Manual Number: 16**

## **Instrument: Water Bath**

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## **WATER BATH**

### Switch ON

Step 1: Connect the main power cord to the switch board.

Step 2: Turn ON the power switch, followed by the mains switch.

Step 3: Set the water bath at the required temperature.

### Switch OFF

Step 1: Turn OFF the mains switch on the instrument.

Step 2: Turn OFF the power switch on the main supply.

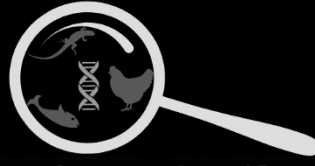
### CLEANING

Ensure that the coil remains submerged in water always and also timely replacement of the water (RO).

(PS: DO NOT leave the water bath ON overnight)

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**Working Manual Number: 17**  
**Instrument: T100/ SimpliAmp Thermal  
Cycler**

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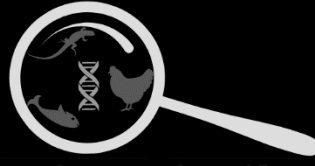
## THERMAL CYCLER

1. Check the heated cover is closed.
2. Power on the instrument. The power switch at the bottom left of the back of the instrument. Wait for the systems to start up, this may take up to 5 minutes.
3. Select "Enter As Guest".
4. Select "Browse/New Methods".
5. Choose your run method in the list or from your USB drive.

If you do not have a run method, select "New" to create a new run method.

Create a new run method

6. Open the heat cover put your PCR tubes/plate into the well. Close the cover carefully.
7. Select "Start run".
8. Enter your "Reaction volume" & "Cover temperature". Select "Do Not Heat Cover" if you do not need it.
9. Select "Start Run Now". The system will start the PCR run.
10. When the PCR is running, you can monitor, pause and stop your PCR run using the screen.
11. After the PCR run finish, the instrument's screen will show the run report.
12. Open the heat cover. Remove your tubes/plate out of the instrument.
13. Select back to main menu.
14. Select back to login options.
15. Select to switch off the instrument.
16. Power off the system instrument. The power switch at the bottom left of the back of the instrument.
17. Sign the log sheet before you leave.



DIVISION OF DEVELOPMENTAL BIOLOGY & TOXICOLOGY

# **Working Manual Number: 18**

## **Instrument: LC96 Light Cycler Roche**

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## LIGHT CYCLER 96

### 3 Programming the experiment with the LightCycler® 96 Instrument Software

The information provided in the experiment definition controls the LightCycler® 96 Instrument during an experiment run. The experiment definition specifies the target temperatures and hold times of the thermal block cycler, the number of cycles being executed, and other parameters.

For programming the experiment with the instrument software, the LightCycler® 96 Instrument must be started.

For a comprehensive description of the LightCycler® 96 Instrument Software, refer to the LightCycler® 96 System Operator's Guide, chapter LightCycler® 96 Instrument Software.

In addition, the help browser provides information on the currently open tab of the LightCycler® 96 Instrument Software.

To program an experiment:

- Create a new experiment, see section Creating the experiment, below.
- Add one or more programs and define the temperature profile for each step of a program, see section

Creating the temperature profile, on page 35.

- Specify the reaction volume and the detection format for the experiment, see section Configuring the detection format and reaction volume, on page 40.

#### 3.1 Creating the experiment

This user training guide describes how to generate a completely new experiment. For a detailed description of all options for creating an experiment with the LightCycler® 96 Instrument Software, refer to the LightCycler® 96 System Operator's Guide, chapter C, section Experiments.

In the global action bar of the instrument software main window, choose New.

The Create New Experiment window area opens.

Programming the experiment with the LightCycler® 96 Instrument Software

Creating the experiment

Choose New empty experiment to create a new, empty experiment. The new experiment has the default name New\_Experiment.

Choose the Experiment Name field.

A keyboard dialog box opens.

Programming the experiment with the LightCycler® 96 Instrument Software

Creating the experiment

In the New Experiment Name field, specify the name for the new experiment using the keys, and close the dialog box with OK.

In the Create New Experiment window area, choose Create.

The LightCycler® 96 Instrument Software performs the following steps:

- It adds the new experiment to the list in the OVERVIEW tab.
- It opens the Run Editor tab for the new experiment.

Programming the experiment with the LightCycler® 96 Instrument Software

Creating the temperature profile

### 3.2 Creating the temperature profile

For detailed information on the applicable values for the experiment run parameters, see the corresponding section in the chapter Main applications, on page 57.

You can only edit a program, and thus also a profile, as long as no run has been performed.

To create a temperature profile:

- Add one or more new programs to the temperature profile and specify the cycling sequence, see section To add a new program and specify the number of cycles, below.
- Define the temperature profile for each step of a program, see section To specify the temperature profile for each step of a program, on page 37.

To add a new program and specify the number of cycles

Select one of the available programs and choose Add.

The program is added to the program list on the Profile tab.

Programming the experiment with the LightCycler® 96 Instrument Software

Creating the temperature profile

In the program list, choose the new program. Then choose the pencil button.

The Program Settings window area opens.

Optional: In the Name field, specify the name for the selected program.

If necessary (for example, for an amplification program) specify the number of repeats of the selected program (cycles).

Possible values: 1 to 99

For detailed information on the applicable VALUES for the number of cycles, see the corresponding section in the chapter Main applications, on page 57.

Choose Back to apply your settings to the selected program.

The Program Settings window area is closed. The program list is displayed with the changed settings.

Optional: Repeat steps 1 to 7 to add further programs to your profile and specify the corresponding number of cycles if necessary.

Programming the experiment with the LightCycler® 96 Instrument Software

Creating the temperature profile

To specify the temperature profile for each step of a program

A step can only be edited as long as no run has been performed.

For a comprehensive description of all options of the LightCycler® 96 Instrument Software, refer to the LightCycler® 96 System Operator's Guide, chapter LightCycler® 96 Instrument Software.

In the step list, select a step and choose the pencil button.

The Step Settings window area opens.

Programming the experiment with the LightCycler® 96 Instrument Software

Creating the temperature profile

In the Temperature window area, choose the corresponding field one after the other to specify the de-

fault values of the following parameters for the selected step:

Ramp (°C/s):

Maximum value for heating: 4.4°C/s Maximum value for cooling: 2.2°C/s

Duration (s):

Possible values: 1 to 7200 s (=2 h)

Target (°C):

Possible values: 37 to 98°C

For the steps of an amplification program: For Mode, leave the default option (Standard).

For detailed information on the applicable VALUES for the experiment run parameters, see the corresponding section in the chapter Main applications, on page 57.

To specify the listed parameters, proceed as follows for each parameter of the selected step:

Choose the field for each parameter. The corresponding dialog box opens.

In the dialog box, choose the relevant number buttons to specify the applicable value.

Choose OK to apply your setting to the parameter.

Programming the experiment with the LightCycler® 96 Instrument Software

Creating the temperature profile

In the Acquisition Mode window area, choose one of the following options for the selected step.

Single: Applicable for amplification program steps (one measurement/cycle).

Continuous (Readings/°C): Applicable for melting program steps. Also enter in the field the number of optical acquisitions to be performed in the corresponding list.

Default value: 5 Readings/°C Possible values: 1 to 25 Readings/°C

None: Applicable for steps that do not require fluorescence measurement.

For detailed information on the applicable VALUES for the experiment run parameters, see the corre-

sponding section in the chapter Main applications, on page 57.

Choose Back to apply your settings to the corresponding parameters.

View the temperature profile area for a graphical representation of the entire experimental protocol you have defined.

Programming the experiment with the LightCycler® 96 Instrument Software

Configuring the detection format and reaction VOLUME

### 3.3 Configuring the detection format and reaction volume

You cannot change or customize the detection format definition after the run has started.

To complete the run definition:

Specify the dye-specific parameters for the detection format, see section To specify the detection format for the experiment, below.

Specify the reaction volume, see section To specify the reaction volume for the experiment, on page 42.

The LightCycler® 96 Instrument Software automatically saves all changes in the experiment file on the instrument.

To specify the detection format for the experiment

The detection format specifies one or more excitation-emission filter combinations (detection channels) suitable for your experiment.

For detailed information on the applicable values for the detection format, see the corresponding section in the chapter Main applications, on page 57.



For a comprehensive description of all options of the LightCycler® 96 Instrument Software, refer to the LightCycler® 96 System Operator's Guide, chapter LightCycler® 96 Instrument Software.

Choose the pencil button next to the Detection Format list.

The Detection Format window area opens.

Programming the experiment with the LightCycler® 96 Instrument Software

Configuring the detection format and reaction VOLUME

Choose the tab of the detection channel you want to use.

In the Selected column, choose no more than one dye per detection channel, to specify that the corresponding channel is to be used.

Only one dye can be selected per channel. The software automatically deselects a dye if you try to select more than one dye in the same channel group.

For Quant Factor and Melt Factor, leave the default values.

For Integration Time [s], leave the default value (Dynamic).

Repeat steps 3 and 4 to specify another detection channel for your detection format.

You cannot combine SYBR Green I or the ResoLight dye with any dye of any other channel.

Programming the experiment with the LightCycler® 96 Instrument Software

Configuring the detection format and reaction VOLUME

To specify the reaction volume for the experiment

In the General window area, choose the Reaction Volume ( $\mu$ l) field. A dialog box for specifying the value opens.

Specify the applicable reaction volume to be used in your experiment.

The LightCycler® 96 Instrument supports reaction volumes from 10 to 50  $\mu$ l.

For detailed information on the applicable VALUE for the reaction VOLUME, see the corresponding section in the chapter Main applications, on page 57.

As the LightCycler® 96 Instrument does not VALIDATE the reaction VOLUME, ensure that the specified reaction VOLUME matches the VOLUME pipetted into the wells of the multiwell plate.

To save the experiment

The LightCycler® 96 Instrument Software automatically saves all changes in the experiment file on the instrument.

The LightCycler® 96 Instrument Software supports the following experiment file types:

- \*.lc96p (LightCycler® 96 experiment files for processed experiments)
- \*.lc96u (LightCycler® 96 experiment files for unprocessed experiments)

For detailed information on saving in the instrument software, refer to the LightCycler® 96 System Operator's Guide, chapter LightCycler® 96 Instrument Software.

Running the experiment

Starting the run

#### 4 Running the experiment

After defining the setup parameters (temperature profile, reaction volume, and detection format), and saving the definition, you are ready to run the LightCycler® 96 experiment.

For starting an experiment run, the experiment must be transferred to the LightCycler® 96 Instrument. An experiment run can only be started on the instrument using the

LightCycler® 96 Instrument Software. For detailed information on how to transfer an experiment to the instrument, see section Transferring the experiment to the instrument, on page 30.

During an experiment run, it is not recommended to use a USB drive, for example, for exporting or importing data, or for synchronizing an experiment, as this may cause problems in the measurement process.

##### 4.1 Starting the run

To start the experiment run

Before loading the multiwell plate into the LightCycler® 96 Instrument, it must be sealed with the self-adhesive sealing foil. Use the sealing foil applicator provided with the instrument for proper sealing.

Always centrifuge the filled and sealed plate before loading it into the instrument. For detailed information, see the corresponding section in the chapter Main applications, on page 57.

For detailed information on how to set up the samples, see the corresponding section in the chapter Main applications, on page 57. For comprehensive information, refer also to the LightCycler® 96 System Operator's Guide, chapter "System description".

Load the LightCycler® 480 Multiwell Plate 96 with the samples into the LightCycler® 96 Instrument.

Ensure that the temperature profile of the experiment is specified correctly, for example, by establishing a validation process. For detailed information, see section Creating the temperature profile, on page 35.

## Running the experiment

### Starting the run

In the global action bar of the LightCycler® 96 Instrument Software main window, choose Start.

B Choose the Raw Data tab to view the progress of the running experiment.

### Running the experiment

#### Monitoring the run

#### 4.2 Monitoring the run

If the LightCycler® 96 Instrument and the computer running the

LightCycler® 96 Application Software are not connected to a network, an experiment run can only be monitored on the instrument using the LightCycler® 96 Instrument Software.

To monitor the experiment run

For a detailed description of the charts on the 'Raw Data' tab, refer to the

LightCycler® 96 System Operator's Guide, chapter LightCycler® 96 Instrument Software.

On the Raw Data tab, choose the Temperature tab to monitor the summary of the programs selected for the experiment and their temperature and time settings in real time.

Choose one of the <dye> tabs, to monitor the relevant fluorescence curves, that is, the fluorescence intensity against the time in hours, minutes, and seconds for the entire run in real time. There is one curve for each sample that has a gene labeled with the selected dye.

Running the experiment

Monitoring the run

For amplification programs only:

- In the toggle button, select Fluorescence Heat Map. The heat map for the selected dye is displayed.
- Monitor the heat map for the selected dye.

B

Run finished

The end of a run is indicated as follows:

- The status bar shows the instrument status Ready.
- On the Overview tab, the icon in the Status column changes to (Executed).
- The LightCycler® 96 Instrument unlocks the loading module.
- In the experiment progress window area, the start time and the end time are shown.
- The Raw Data tab provides the final raw data.

Transferring the experiment from the instrument to the application software

5 Transferring the experiment from the instrument to the application software

After the experiment run, the raw data gathered by the instrument software must be transferred to the application software for analysis.

The LightCycler® 96 Instrument Software automatically saves all changes in an experiment file. The experiment file is saved according to its original location:

- On the LightCycler® 96 Instrument.
- On the USB drive.
- On both media if the operator has synchronized the storage locations.

For detailed information on synchronizing, refer to the help browser of the LightCycler® 96 Instrument Software.

To transfer an experiment file including the raw data from the instrument to the application software:

When the run is finished, save the experiment file including the raw data to a USB drive, see section

To save the experiment raw data to the USB drive, below.

Transfer the stored data to a computer on which the LightCycler® 96 Application Software is in- stalled. Open the experiment in the application software for data analysis, see section To transfer the stored data to the application software, on page 49.

To save the experiment raw data to the USB drive

This procedure is optional. You only have to save the raw data to the USB drive if it is only saved on the instrument.

Insert a USB drive into the USB interface on the right side of the instrument.

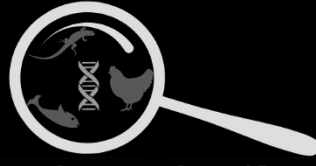
Wait until the USB icon is displayed in the status bar of the instrument software.

Transferring the experiment from the instrument to the application software

On the OVERVIEW tab, select the experiment you want to transfer to the application software.

Choose Synchronize to store the experiment on the USB drive.

In the Storage column of the selected experiment, the Synchronized icon is shown.



DIVISION OF DEVELOPMENTAL BIOLOGY & TOXICOLOGY

# **Working Manual Number: 19**

## **Instrument: pH Meter**

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## Principles of operation of a pH meter

A pH meter is essentially a voltmeter with a high input impedance which measures the voltage of an electrode sensitive to the hydrogen ion concentration, relative to another electrode which exhibits a constant voltage. The key feature of the pH-sensitive electrode is a thin glass membrane whose outside surface contacts the solution to be tested. The inside surface of the glass membrane is exposed to a constant concentration of hydrogen ions (0.1 M HCl).

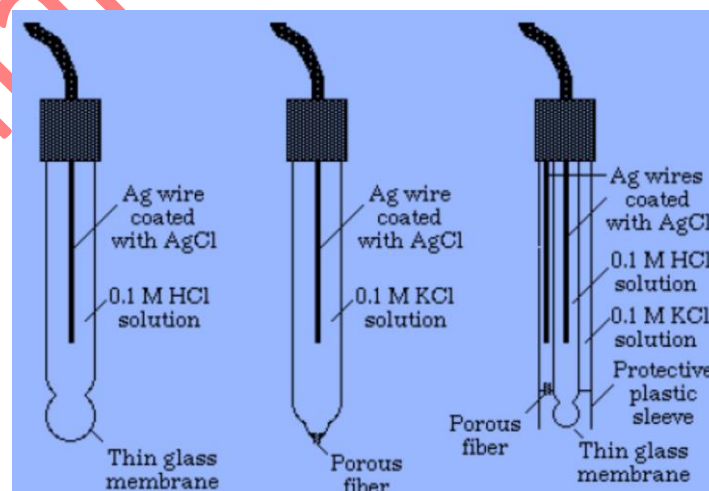
Inside the glass electrode assembly, a silver wire, coated with silver chloride and immersed in the HCl solution, is called an Ag/AgCl electrode. This electrode carries current through the half-cell reaction. The potential between the electrode and the solution depends on the chloride ion concentration, but, since this is constant (0.1 M), the electrode potential is also constant.

A reference electrode is needed to complete the electrical circuit. A common choice is to use another Ag/AgCl electrode as the reference. The Ag/AgCl electrode is immersed in an 0.1 M KCl solution which contacts the test solution through a porous fiber which allows a small flow of ions back and forth to conduct the current. The potential created at this junction between the KCl solution, and the test solution is nearly zero and nearly unaffected by anything in the solution, including hydrogen ions.

Using the pH Meter: Allow the meter a few minutes to stabilize after you plug it in. When you are not using the meter, keep the electrode immersed in pH 7.0 buffer to a depth of about one inch. The meter must be calibrated by using standards of known pH before an unknown is measured. Since the unknowns are acidic, the pH 4.00 and pH 7.00 standards should be used.

An accurate pH reading depends on standardization, the degree of static charge, and the temperature of the solution.

Glass electrode      Reference electrode      Combined electrode



1. The pH meter should be standardized each time it is used with a buffer of known pH, preferably one closest to the desired final pH. To calibrate the pH meter, expose the hole in the electrode, rinse the electrode with deionized water, and place the electrode in a standard solution, e.g., pH 7. Turn the selector to "pH". Adjust the pH meter to the appropriate pH. Rinse electrode with deionized water and place in a second standard buffer solution. The choice of the second standard depends on the final the standard pH buffers used should be 7 and 10. If the final pH desired, for example, if the final pH desired is 8.5, t pH desired is 5.5, the standard pH buffers used should be 4 and 7. Turn the selector to "pH". Adjust the temperature knob to the second standard pH. Rinse the electrode with deionized water and return the electrode to the soaking solution.
2. When rinsing the electrode, never wipe the end, but blot gently since wiping can create a static electric charge, which can cause erroneous readings.
3. Make sure the solution you are measuring is at room temperature since the pH can change with a change in temperature.
4. The pH-sensitive glass membrane is very thin and very easily broken. Do not touch the membrane with anything harder than a Kim-Wipe and do that very gently. Do not drop the electrode or bump it on the bottom of the beaker when immersing it in a solution.
5. The glass membrane must be thoroughly hydrated to work properly. Do not allow the electrode to remain out of water any longer than necessary. When the electrode is not in use, keep it immersed in the pH 7.00 buffer. Do not put the electrode down on the desk.

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