Automated Protein Synthesizer

Protemist DT II

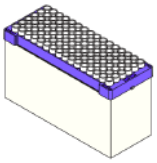
Instruction Manual
- Quick Reference -

CellFree Sciences Co., Ltd.

Table of Contents

1. Consumables

Following items are necessary for Protemist® DT II

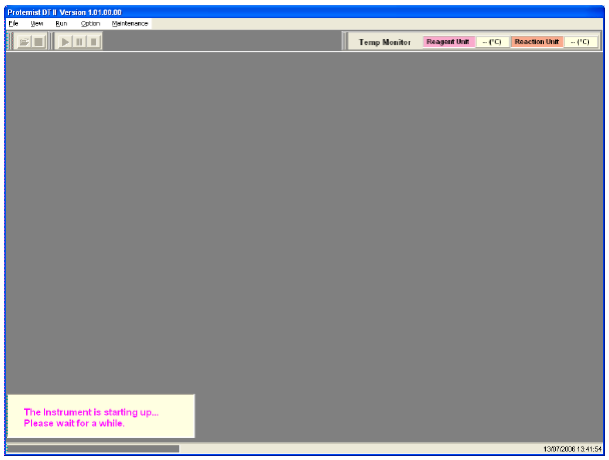
Style	Product name	Specification	Remarks
	Package tip, 1,000 ul	CellFree Sciences Co., Ltd. Catalog No. DIP-1000V-PT24 96 tips/set 24 set/case	Reagent pipet
	Bulk tip, 1,000 ul	CellFree Sciences Co., Ltd. Catalog No. APS-DIP-1000V 500 tips/bag 2 bags/case	Reagent pipet
	Reaction cup	CellFree Sciences Co., Ltd. Catalog No. DT2-P01 60 cups/case	For translation and purification reaction of 6-ml scale
	Micro tubes 2.0 ml, Type I, with skirted base, neutral cap	SARSTEDT Catalog No. 72.694.005 100/bag, 10 bags/case	Container for template DNA of 6-ml scale, transcription mixture, translation mixture, solution A, and product recovery
	50 ml high-clarity Polypropylene conical centrifuge tube	BD-Falcon Catalog No. 352070 25 pieces/bag 20 bags/case	Container for translation buffer and wash buffer
	14 ml, 17 x 100 mm Polypropylene round-bottom test tube	BD-Falcon Catalog No. 352059 25 pieces/bag 20 bags/case	Container for elution buffer, resin, and flow through fraction
	PCR strip tubes, 1 x 8, 0.2 ml	Greiner-bio-one Catalog No. 673210 125 pieces/bag	Container for template DNA of 1.2-ml scale
	24-well cell culture plate Nontreated polystyrene, flat-bottom with lid	BD-Falcon Catalog No. 351147 50 trays/case	Container for translation reaction of 1.2-ml scale
	Square bottle, 60 ml	Nalge Nunc International Catalog No. 2016-0060 12 pieces/package 6 packages/case	Liquid waste bottle

2. Set up the Instrument

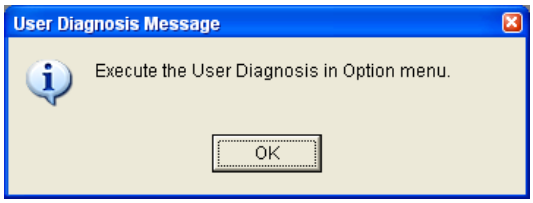
2-1. Start up the Instrument and the software

Note: Before one or more hours to set up reagents, turn power on the Instrument and the control PC, then start the software so that the Reagent Unit is automatically cooled to and controlled at 4℃.

1. Turn power on the Instrument.
2. Turn power on the control PC connected to the Instrument.
3. Double click **Protemist DT II** icon on the screen to start the software. Initialization of the Instrument will be performed.



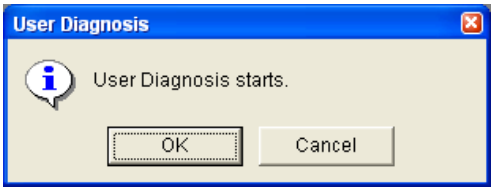
4. **User Diagnosis Message** window is displayed after the initialization is completed.



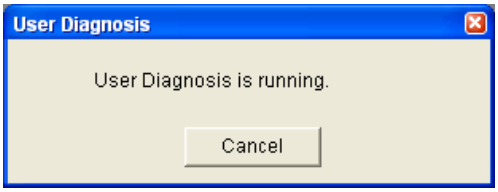
5. Click **OK** in **User Diagnosis Message** window.

2-2. Perform self diagnosis

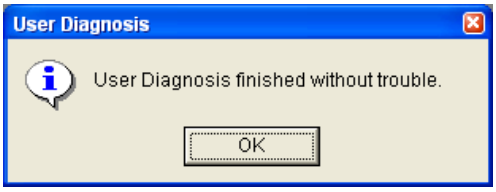
1. Select **User Diagnosis** from **Option** menu. **User Diagnosis** window is displayed.



2. Click **OK** to begin self diagnosis. The following window is displayed while self diagnosis is running.



3. Self diagnosis is completed when the following window is displayed.



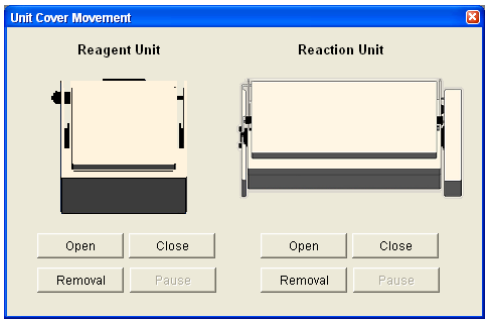
4. Click **OK** in the window.

2-3. Set up Reagent Unit and Reaction Unit

Handle the Units and accessories with hand gloves to avoid RNase contamination.

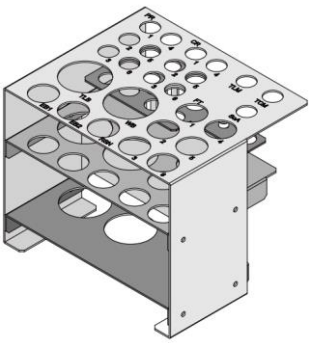
Reagent Unit

1. Select **Unit Cover Movement** in **Option** menu. **Unit Cover Movement** window will be displayed.

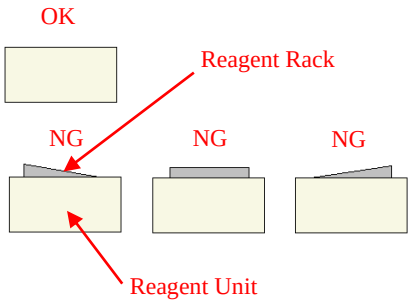


2. Click **Open** of the Reagent Unit in the **Unit Cover Movement** window to open the Reagent Unit Cover.
3. Open the Safety Cover of the Instrument.
4. Install the Reagent Rack in the Reagent Unit.

   ***Caution*:** Ensure the Reagent Rack does not tilt in the Reagent Unit.

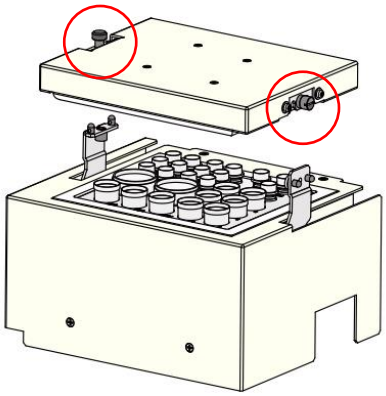


Reagent Rack

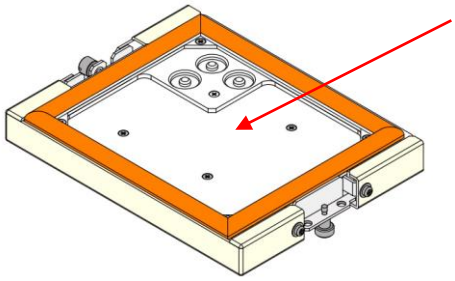


5. Close the Safety Cover of the Instrument.
6. Click **Removal** of the Reagent Unit in the **Unit Cover Movement** window to lift the Reagent Unit Cover.
7. Open the Safety Cover of the Instrument.

8. Remove the Reagent Unit Cover by unscrewing two screws on both ends of the Reagent Unit Cover shown below.



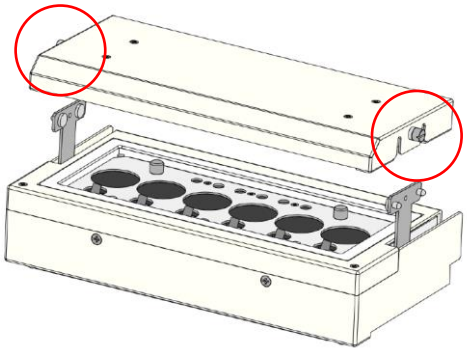
9. Wipe the back of the Cover first with a paper towel infiltrated by RNase decontaminant, and then wipe several times with another paper towel infiltrated by nuclease free water.



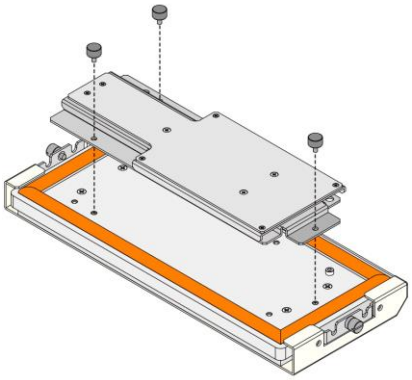
10. Replace the Cover to the Reagent Unit and fasten it by turning the screws on both ends of the Cover.
11. Close the Safety Cover of the Instrument.
12. Click **Close** of the Reagent Unit in the **Unit Cover Movement** window to restore the temperature of the unit at 4℃.

Reaction Unit

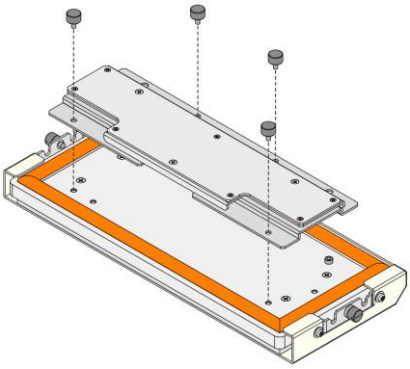
1. Close the Safety Cover of the Instrument.
2. Select **Unit Cover Movement** in **Option** menu. **Unit Cover Movement** window will be displayed.
3. Click **Removal** of the Reaction Unit in the **Unit Cover Movement** window to lift the Reaction Unit Cover.
4. Open the Safety Cover of the Instrument.
5. Remove the Reaction Unit Cover by unscrewing two screws on both ends of the Reaction Unit Cover shown below.



6. Place the Inner Cover for 1.2-ml or 6-ml scale, as the case may be, by unscrewing the screws on the Reaction Unit Cover. The Inner Cover is fastened with three screws for 1.2-ml scale and four screws for 6-ml scale, respectively, as shown below.



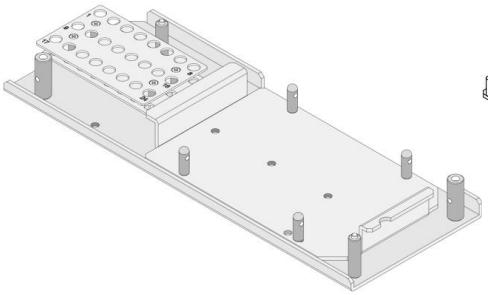
Inner Cover for 1.2-ml scale



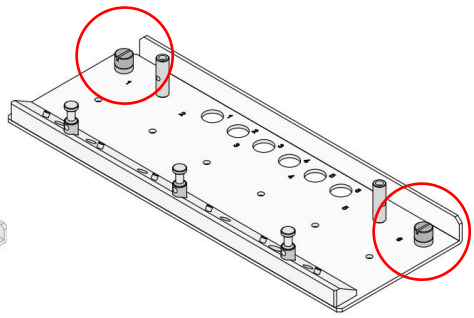
Inner Cover for 6-ml scale

7. Wipe the surface of the Inner Cover first with a paper towel infiltrated by RNase decontaminant, and then wipe several times with a paper towel infiltrated by nuclease free water.
8. Replace the Reaction Unit Cover to the Reaction Unit and fasten it by turning the screws on both ends of the Cover.
9. Close the Safety Cover
10. Click **Open** of the Reaction Unit in the **Unit Cover Movement** window.

11. Install the Reaction Adapter Base for 1.2-ml or 6-ml scale, as the case may be, in the Reaction Unit. The Reaction Adapter Base for 6-ml scale is fastened to the Reaction Unit by turning two screws as shown below.



Reaction Adapter Base for 1.2-ml scale



Reaction Adapter Base for 6-ml scale

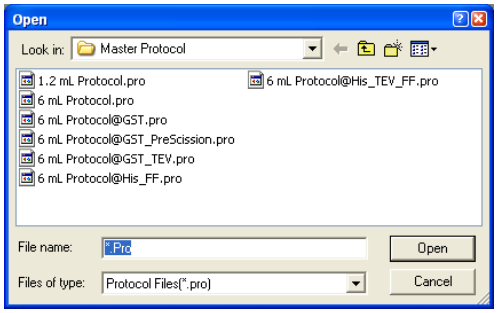
12. Close the Safety Cover of the Instrument.
13. Click **Close** of the Reaction Unit in the **Unit Cover Movement** window.
14. Close the **Unit Cover Movement** window.

2-4. Select protocol

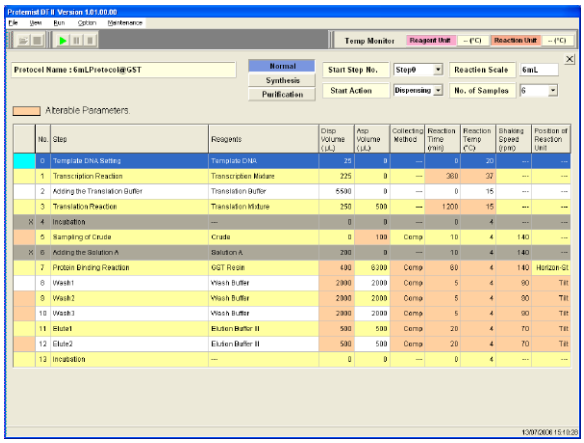
Seven default protocols are installed in the control PC. Use the following list to choose an appropriate protocol for your purpose.

Translation scale	Protein synthesis	Purification	Tag	Elution method	Protocol name
1.2 ml	Yes	No	-	-	1.2 ml protocol
6 ml	Yes	No	-	-	6 ml protocol
	Yes	Yes	GST	Reduced glutathione	6 ml protocol@GST
				TEV protease	6 ml protocol@GST_TEV
				PreScission protease	6 ml protocol@GST_PreScission
	Yes	Yes	His	Imidazole	6 ml protocol@His_FF
				TEV protease	6 ml protocol@His_TEV_FF

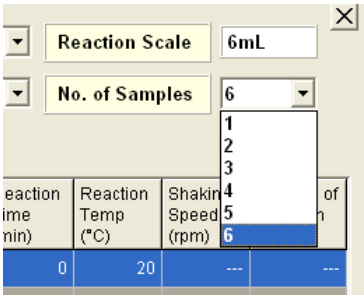
1. Select **Open** in **File** menu. **Open** window will be displayed.



2. Find the folder that contains the protocol file to be used. The default protocol files are contained in the folder named **Master Protocol**.
3. Select the protocol then click **Open** in the window. The protocol will be displayed on the screen.



4. Select **Number of Samples** in the pull down list.

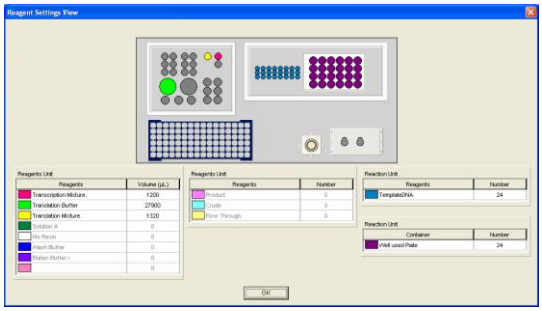


***Caution*:** DO NOT change the parameters that have been present in the columns **Collecting Method** and **Position of Reaction Unit**.

2-5. Preparation of the reagents

Confirm the volume of reagents to be prepared.

1. Select **Reagent Settings View** from **View** menu. **Reagent Settings View** window will be displayed. Confirm the kind of reagents and their volume to be prepared.



Note: The names of reagents in the Reagent Preparation Manual are different from those in the **Reagent Settings View** window as shown in the table below. Please refer to the Reagent Preparation Manual for the details of reagent preparation.

Reagent name shown in the Reagent Settings View	**Reagent name in Reagent Preparation Manual**
Elution buffer I	Elution buffer B
	Elution buffer C
	Elution buffer F
Elution buffer II	Elution buffer D
	Elution buffer G
Elution buffer III	Elution buffer A
	Elution buffer E

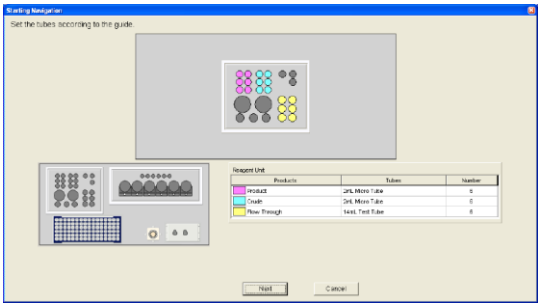
2. Click **OK** in **Reagent Settings View** window to close the window.
3. Prepare the reagents as described in the Reagent Preparation Manual.

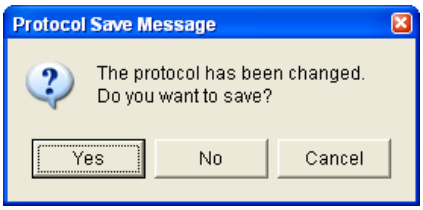
***Caution*:** The volume of each reagent should be equal to or more than them the one indicated in **Reagent Settings View** window. Otherwise, the Instrument may pause during the process.

2-6. Installation of the reagents and other Items

Reagent Unit

1. Click on **Run** menu, place the pointer on **Start/Restart** in the **Run** menu, and select **Starting Navigation**. **Starting Navigation** window will be displayed. If **Protocol Save Message** window appears, click **Yes** in the window if you want to save the change in the protocol. Enter a file name if prompted.

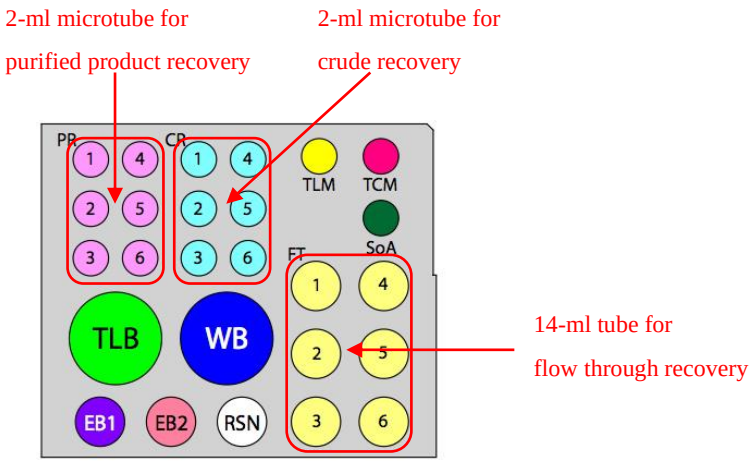




Note: If changes are made in default protocols, save those protocols with **Save As** under new file names.

2. Close the Safety Cover of the Instrument.
3. Select **Unit Cover Movement** in the **Option** menu.
4. Click **Open** of the Reagent Unit in the **Unit Cover Movement** window and have the Unit Cover open. Do the same for the Reaction Unit.
5. Close the **Unit Cover Movement** window.

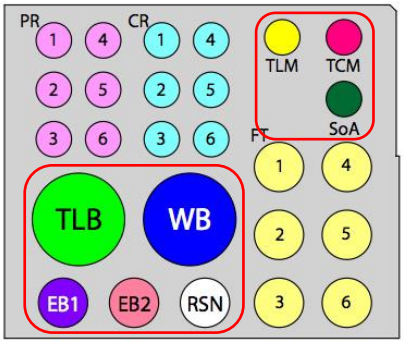
6. To perform purification, install recovery tubes without caps in the Reagent Unit as indicated in the **Starting Navigation** window.



Reagent Rack in Reagent Unit

7. Click **Next** in the **Starting Navigation** window.
8. Remove the caps from the tubes containing reagents and install them in the Reagent Unit as indicated in the **Starting Navigation** window. Some reagent names that appear in the **Starting Navigation** window are different from those used in the Reagent Preparation Manual. Use the following table as the cross reference.

Reagent name in Reagent Preparation Manual	Reagent name shown in the window	Position on the Reagent Rack
Translation mixture	Translation mixture	TLM
Transcription mixture	Transcription mixture	TCM
Solution A	Solution A	SoA
Translation buffer	Translation buffer	TLB
Wash buffer A	Wash buffer	WB
Wash buffer B		
Elution buffer A	Elution buffer III	EB1
Elution buffer E		
Elution buffer B	Elution buffer I	
Elution buffer C		
Elution buffer F		
Elution buffer D	Elution buffer II	EB2
Elution buffer G		
GST resin	GST resin	RSN
His resin	His resin	



Reagent Rack in Reagent Unit

9. Close the Safety Cover of the Instrument.
10. Select **Unit Cover Movement** in the **Option** menu.
11. Click **Close** of the Reagent Unit in the **Unit Cover Movement** window.

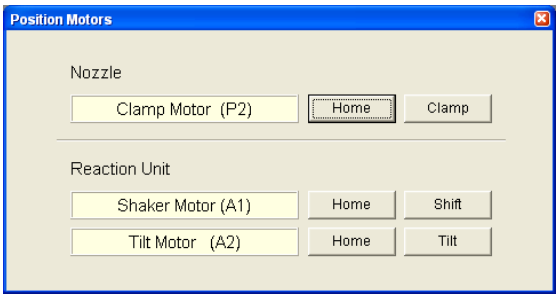
    Note: Be sure to close the Cover of the Reagent Unit to keep the reagents at 4℃.

12. Close the **Unit Cover Movement** window.

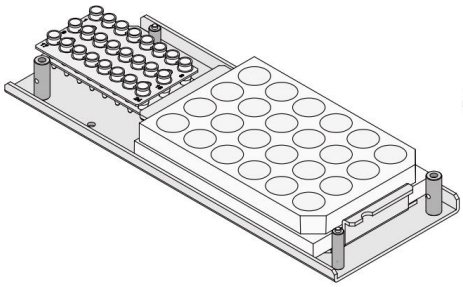
Reaction Unit

Note: Before carrying out the following steps, wipe the Reaction Adapter Cover first with a paper towel infiltrated by RNase decontaminant, and then wipe several times with a paper towel infiltrated by nuclease free water.

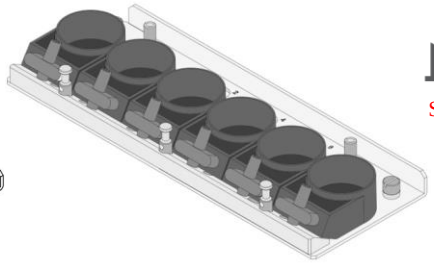
1. Click **Next** in the **Starting Navigation** window.
2. Close the Safety Cover of the Instrument.
3. Select **Position Motors** in the **Option** menu. **Position Motors** window will be displayed.



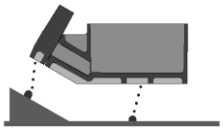
4. Click **Tilt** of the Tilt Motor (A2) in the **Position Motors** window. Reaction Unit will be tilted.
5. Close **Position Motors** window
6. Install a 24-well plate for 1.2-ml scale or as many as 6 reaction cups for 6-ml scale in Reaction Unit as indicated in the **Starting Navigation** window.



24-well plate

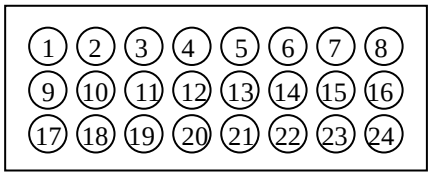


Reaction cups

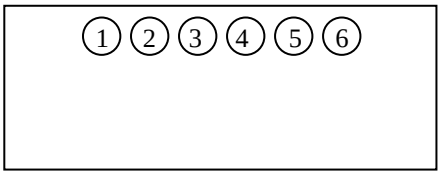


Setting the reaction cup
(vertical section view)

7. Click **Next** in the **Starting Navigation** window.
8. As shown in the **Starting Navigation** window, install in the Reaction Unit 0.2-ml strip microtubes without caps for 1.2-ml scale or 2-ml microtubes without caps for 6-ml scale, those micritubes containing template DNA.

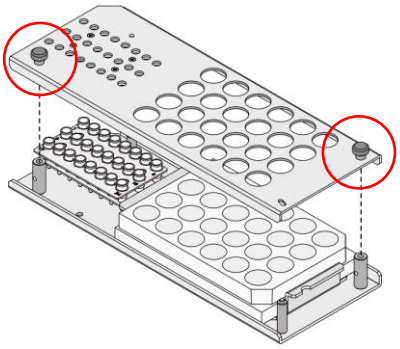


Sample number for 1.2-ml scale

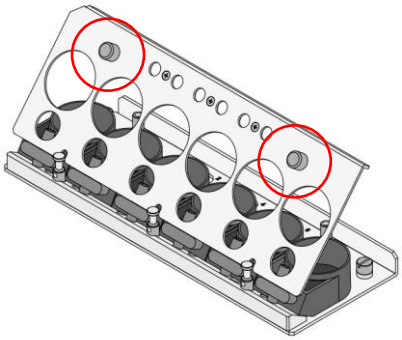


Sample number for 6-ml scale

9. Close the Safety Cover of the Instrument.
10. Select **Position Motors** in the **Option** menu. **Position Motors** window will be displayed.
11. Click **Home** of the Tilt Motor (A2) in the **Position Motors** window. Reaction Unit will return to home position.
12. Close **Position Motors** window.
13. Install the Reaction Adapter Cover for 1.2-ml or 6-ml scale and tighten the screws on it to fasten the Cover to the Reaction Adapter Base in the Reaction Unit.



Reaction Adapter Cover for 1.2-ml scale



Reaction Adapter Cover for 6-ml scale

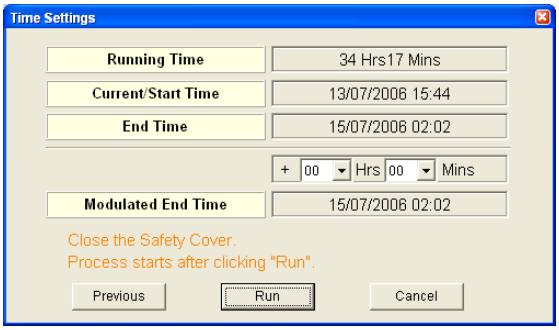
14. Close the Safety Cover of the Instrument.
15. Select **Unit Cover Movement** in the **Option** menu.
16. Click **Close** of the Reaction Unit in the **Unit Cover Movement** window to close the Reaction Unit Cover.
17. Close the **Unit Cover Movement** window.

Installation of tips, waste bottle, and dust box

1. Click **Next** in the **Starting Navigation** window. **Starting Preview** window will appear for final confirmation before proceeding further.
2. As indicated in the **Starting Preview** window, completely fill the tip rack with tips, install an empty waste bottle, and an empty dust box.
3. Close the Safety Cover of the Instrument.

2-7. Start the process

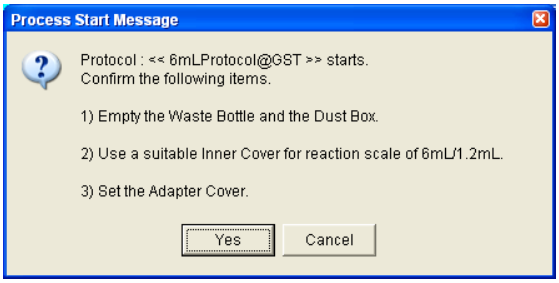
1. Click **Go to…** in the **Starting Preview** window. **Time Settings** window will be displayed.



2. Enter additional time to modulate the End Time if necessary.

   Note: Modulated End Time will be reflected in the incubation time after the translation step to keep the product solution at 4℃. This function is not available for 1.2-ml scale.

3. Click **Run** in the **Time Settings** window. **Process Start Message** window is displayed.



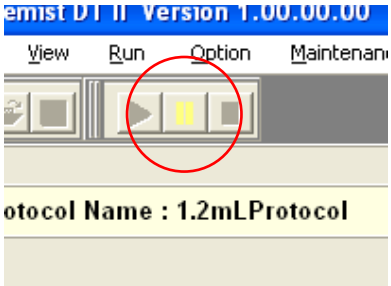
4. Click **Yes** in the **Process Start Message** window to start the process.

3. Pause or Abort the Process

If the process is required to be paused or aborted, follow the procedure shown below.

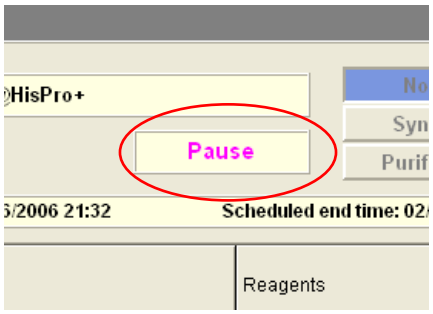
3-1. Pause the process

1. Click on **Pause** button in the process monitoring window.



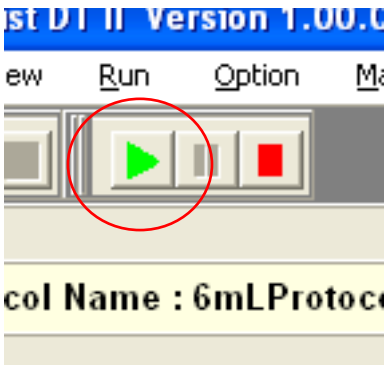
2. **Pause** will be displayed in the status column.

***Caution*:** The Instrument does not stop right away when the **Pause** button is clicked on. Before opening the Safety Cover, confirm the Instrument movement is completely stopped.



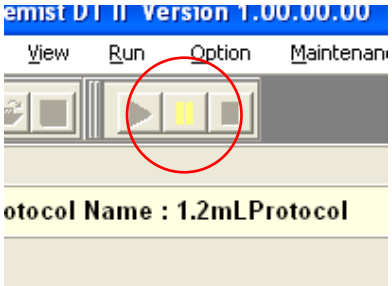
3-2. Restart the process

Click on **Start/Restart** button in the window.

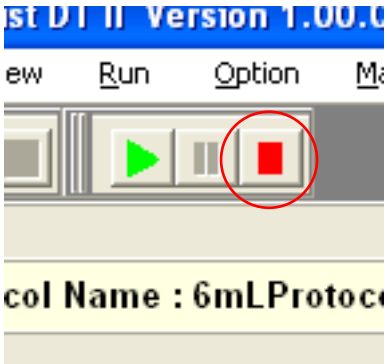


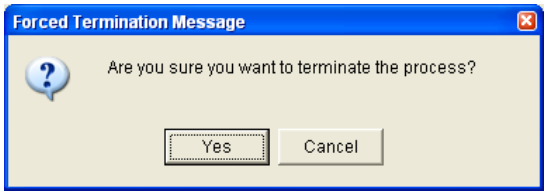
3-3. Abort the process

1. Click on **Pause** button in the window.



2. Click on **Stop** button. **Forced Termination Message** window is displayed.

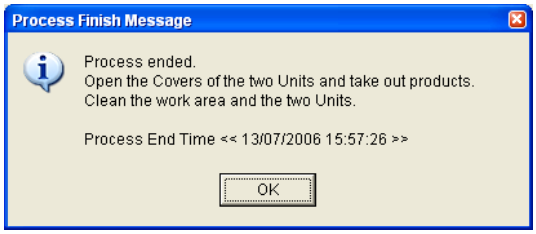




3. Click **Yes** in the **Forced Termination Message** window to terminate the process. The Instrument starts initialization.

   ***Caution*:** Do not open the Safety Cover until the initialization is finished.

4. When the initialization is finished, **Process Finish Message** window will be displayed.

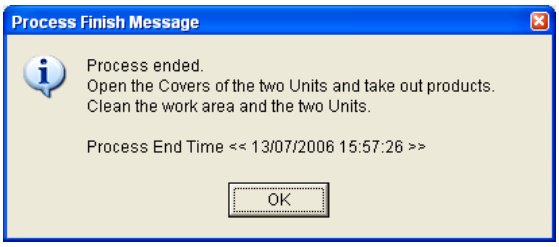


5. Click **OK** in the **Process Finish Message** window.

4. After the Process

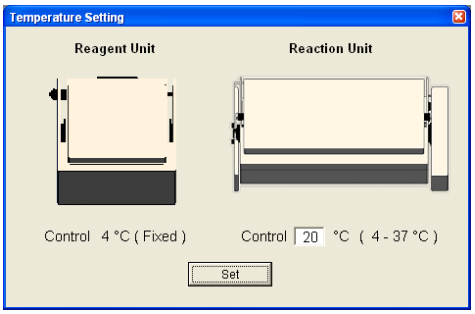
4-1. Take out the products

1. **Process Finish Message** window will be displayed when the process is finished. As long as the **Process Finish Message** window is displayed, the temperature of the Reaction Unit is maintained at 4℃.



2. Click **OK** in the **Process Finish Message** window. The window will be closed. Take out the products quickly.

   Note: The temperature of the Reaction Unit after **OK** is clicked on starts to revert to the one set in the **Temperature Setting** window of **Temp Setting** in the **Option** menu, while the temperature of the Reagent Unit is always controlled at 4℃.

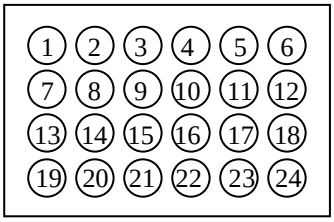


3. Select **Unit Cover Movement** in the **Option** menu.
4. Click **Open** of the Reagent Unit in the **Unit Cover Movement** window and have the Unit Cover open. Do the same for the Reaction Unit.

1.2-ml translation scale without purification

Synthesized products are in a 24-well plate.

1. Remove Reaction Adapter Cover by loosening two screws.
2. Carefully take out the 24-well plate containing synthesized products. Sample numbers on the plate are as shown below



Sample number in 24-well plate

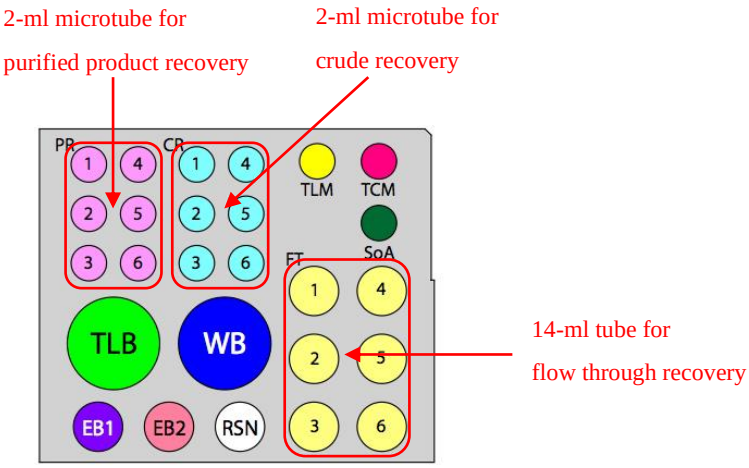
3. Remove the tubes from the Reagent Unit and Reaction Unit.

6-ml translation scale with or without purification

With purification

Purified products are in 2-ml microtubes in the Reagent Unit.

1. Take out the product tubes from the Reagent Unit. The products are located at the positions shown below;

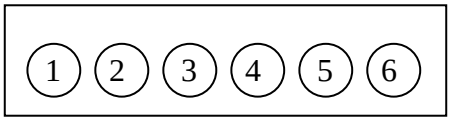


2. Remove the Reaction Adapter Cover by loosening two screws.
3. Take out the tubes from the Reagent Unit and Reaction Unit.

Without purification

Synthesized products are in reaction cups.

1. Remove Reaction Adapter Cover by loosening two screws.
2. Carefully take out the reaction cups containing synthesized products.



Sample number of reaction cups

3. Take out the tubes from the Reagent Unit and Reaction Unit.

4-2. Post-treatment of accessories

Cleaning

Note: Accessories cannot be autoclaved.

1. Remove the Reaction Adapter Base from the Reaction Unit by loosening two screws in the case of the Adapter Base for 6-ml scale.
2. Take out Reagents Rack from Reagent Unit.
3. Close the Safety Cover of the Instrument.
4. Select **Unit Cover Movement** in the **Option** menu.
5. Click **Removal** of the Reagent Unit in the **Unit Cover Movement** window and have the Unit Cover lift. Do the same for the Reaction Unit.
6. Open the Safety Cover of the Instrument.
7. Remove the Reagent Unit Cover and Reaction Unit Cover by loosening the screws.
8. Wipe the following accessories with a paper towel infiltrated by 70% ethanol: Reagent Unit Cover (inside), Reagent Rack, Reaction Unit Inner Cover, Reaction Adapter Cover, Reaction Adapter Base, Reagent Unit (inside), Reaction Unit (inside), and the work area.
9. Replace Reagent Unit Cover and Reaction Unit Cover and turn the screws on the covers to fasten them.
10. Close the Safety Cover of the Instrument.
11. Select **Unit Cover Movement** in the **Option** menu.
12. Click **Removal** of the Reagent Unit in the **Unit Cover Movement** window to lift the Reaction Unit Cover to dry the Unit. Do the same for the Reaction Unit.
13. Close the **Unit Cover Movement** window.

Waste bottle

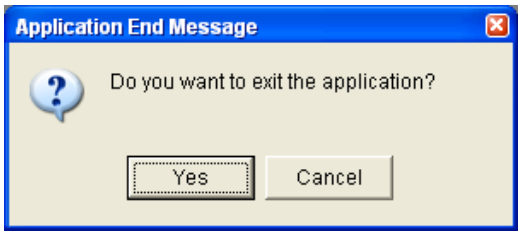
1. Empty the waste liquid bottle and wash it with water and dry it.
2. Replace the bottle in the Instrument.

Dust box

1. Open the dust box container door.
2. Take out the box and empty it.
3. Wash the box and dry it.
4. Replace the box in the Instrument.

4-3. Shut down the system

1. Select **Close** in the **File** menu to close the protocol. If **Protocol Save Message** window is displayed, click **Yes** to save it if necessary.
2. Select **Exit** in **File** menu. **Application End Message** window will be displayed.



3. Click **Yes** to exit the application.
4. Shut down the control PC.
5. Turn off the Instrument.

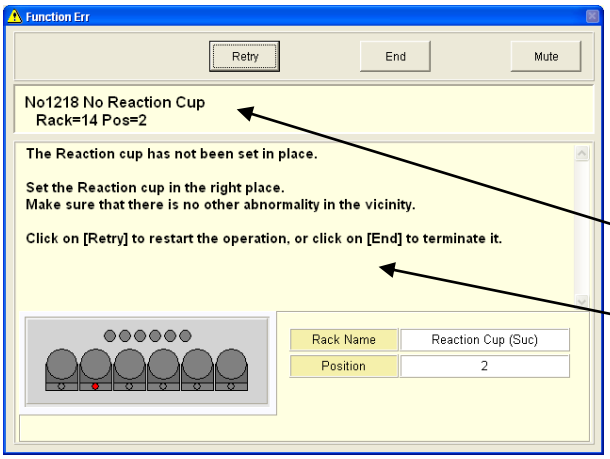
5. Trouble Shooting

When an error occurs in the Instrument, it will send a beep and the **Functional Error** window will be displayed on the screen of the control PC. The content of the error and remedial instruction are described in the **Functional Error** window. Follow the instruction to correct the error. If you could not correct it, contact our technical service with following information.

1. Error number displayed in the **Functional Error** window
2. Log data of the Instrument. The log data is contained in following folder of the PC:
   My computer\\C drive of hard disc\\Protemist DT II\\LOG
   Find the folder of the newest date in the LOG folder, open it, and send us all the files in it.
3. The protocol file that was being used
4. Step number of the protocol at the time of the error
5. Recurrence and frequency of the recurrence of the error
6. Circumstances when the error occurred
7. Version of the software displayed at the top left corner in the application window
8. Serial number of the Instrument on the label attached to the back of the Instrument

5-1. Example of the Functional Error window

When an error occurs, **Functional Error** window will be displayed. Follow the remedial instruction described in the display. If the same error occurs after following the instruction, contact our technical service. An example of the **Functional Error** window is shown below:



[Retry] button: Restart the process.
[End] button: Terminate the process.
[Mute] button: Mute beep sound.

Error number and error message title.

The content of the error and the remedial instruction are described in this field.

To correct the error in the above example, select **Unit Cover Movement** from **Option** menu, click **Open** of the Reaction Unit in **Unit Cover Movement** window to open the Cover, open the Safety Cover, place a Reaction Cup or Cups in the right place(s). After closing the Safety Cover, close the **Unit Cover Movement** window, click on **Retry** button in the **Functional Error** window to restart the process.

5-2. Trouble not accompanied by an error message

Some trouble does not prompt any error message. When such troubles as shown in the table below occur during the operation of the Instrument, try the remedies described in the table. If the same trouble persists, contact our technical service.

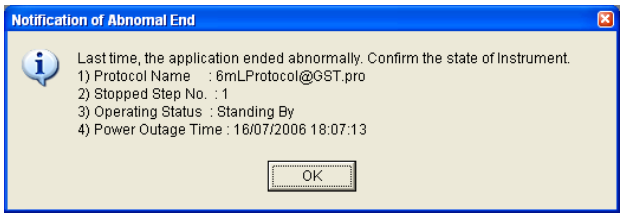
Note: If the battery of the PC becomes empty because of power failure or other causes, error message is not displayed. In this case, follow the procedure in Section 5-3.

Trouble	Possible Cause	Remedies
No working sound from the Instrument when the Instrument is turned on. Power indicator lamp in front of the Instrument does not turn on when the Instrument is turned on.	The power cable is not connected normally.	Confirm that the power cable is connected to the power socket on the rear panel of the Instrument and the other end of the cable is plugged into the wall outlet.
	Circuit breaker of the power source side has shut off the power.	Check the breaker.
	The power fuse in the Instrument is blown.	Contact our technical service.
Abnormal sound, e.g. loud sound.	Obstacles in the work area or the fan is jammed.	Turn off the Instrument and contact our technical service.
The software becomes frozen.	Communication between the Instrument and the control PC is broken. An application software other than Protemist DT II application is started. An external device is connected to the PC.	Refer to Section 5-3.

5-3. Communication error between the Instrument and the PC

When an error occurs in the communication between the Instrument and the PC, follow the procedure described below whether the **Functional Error** window is displayed or not. If the same error persists, contact our technical service.

1. Confirm that the connection between the Instrument and the PC is secured.
2. Turn off and then turn on the Instrument.
3. Restart the PC. If the software is frozen, press Ctrl, Alt, and Del keys of the PC simultaneously to restart Windows XP.
4. After starting Windows XP, double click **Protemist DT II** icon on the screen to start the software. **Notification of Abnormal End** window is usually displayed.



5. Click **OK** in the **Notification of Abnormal End** window. **User Diagnosis Message** window will be displayed.
6. Click **OK** in the **User Diagnosis Message** window.
7. Select **User Diagnosis** from **Option** menu. **User Diagnosis** window is displayed.
8. Click **OK** to begin self diagnosis.
9. Click **OK** in the window after finishing self diagnosis without trouble.

6. Appendix

6-1. Product-related information

All proteins made using our products are for research purpose only; not for use in diagnostic testing and use in human. Contact us for more information and help on the use of our products.

6-2. Intellectual property rights

Our ENDEXT® technologies are covered by US Patent Nos. 6905843, 6869774 and 7919597, and other pending or equivalent patents.

6-3. Trademarks

Company names and product names in this manual are trademarks and registered trademarks of companies. Protemist, ENDEXT, WEPRO, and SUB-AMIX are registered trademarks of CellFree Sciences Co., Ltd..

6-4. Contact us

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