

Revision: ----	Document Code: WI- ML-2433	Document Title: Standard Operating Procedure (SOP) for Eppendorf PCR Thermal Cycler (Model 5333)	 دانشگاه علوم پزشکی و خدمات بهداشتی و درمانی استان خراسان مشهد و نرسکی
--------------------------	--------------------------------------	--	--

1. Purpose

To describe the standard operating procedure for the use of the **Eppendorf Thermal Cycler** (Made in Germany).

2. Scope of Operation

Polymerase Chain Reaction (PCR) is a powerful biochemical technique in biochemistry and molecular genetics that utilizes thermal cycling and enzymes for the rapid and reliable amplification of DNA. It is widely applied in scientific research, disease diagnosis, genetic identification, and various clinical programs.

3. Responsibility

The implementation of this procedure is the responsibility of the relevant laboratory experts.

4. Operating Procedure

A. Editing an Existing Program and Saving it as a New Program

1. Turn on the device (the **Power** switch is located on the rear left side).
2. From the **Main Menu**, use the **Arrow keys** to navigate to **Files** and press **Enter**.
3. Navigate to **Load** and press **Enter**.
4. Select the program you wish to edit and press **Enter**.
 - **Note: If the program list does not appear upon pressing Load and the device only asks to save the previous program, press SEL to toggle from Yes to No, then press Enter. Repeat if necessary.**
5. Navigate to the specific parameter line you wish to modify.
6. Use the **Keypads** to make adjustments and press **Enter** to confirm each change.
 - **Del:** Deletes the line currently indicated by the cursor.
 - **INS:** Inserts a new line above the current cursor position.
7. Once editing is complete, press **Exit**.
8. **Save:** To save the changes, press **Enter** when **Yes** is displayed. If you do not wish to save, press **SEL** to change **Yes** to **No**, then press **Enter**.
9. Rename the file using a unique name and code for identification.

B. Initiating a Run

1. Turn on the device (Power switch on the rear left).
2. Navigate to **Start** and press **Enter**.
3. Select the desired file and press **Enter**.
4. The program will automatically begin the experiment. To view the estimated end time, press **OPT**.
5. **To Stop:** Press **Start/Stop**, then press **Enter**.

C. Setting up a Standard PCR Program

تاریخ اجراء:	تصویب کننده:	تأیید کننده: دکتر محمد رضا عربستانی	تهیه کننده: حسین فضلی
تاریخ بازنگری:	سمت:	سمت: مدیر گروه میکروبیشناسی	سمت: سوپر وایزر آزمایشگاه
شماره صفحه: ۱ از ۲	تاریخ و امضاء:	تاریخ و امضاء:	تاریخ و امضاء:

Revision: ----	Document Code: WI- ML-2433	Document Title: <i>Standard Operating Procedure (SOP) for Eppendorf PCR Thermal Cycler (Model 5333)</i>	 دانشگاه گیلان و خدا بختی و درانی آستان خدا والسلام و برکت
--------------------------	--------------------------------------	--	--

1. Select **Files** and press **Enter**.
2. Select **Standard** and press **Enter**.
3. Use the **Arrow keys** to navigate and the **Keypads** to adjust parameters. Press **Enter** after each modification before moving to the next line. (Refer to the “Editing” section above for adding or deleting lines).
4. **At the end of the program:** Use **Go To** to select the line you wish to loop (typically Step 2, following the initial long denaturation step).
5. **Set Rep:** Select the number of cycles you wish to repeat.
6. **Final Extension:** If required, add a final extension step (this step will not be included in the repeated cycles).
7. **Hold:** Press **SEL** to locate the **Hold** function. Enter the temperature at which you want to hold the samples (typically 22°C).
8. Press **Exit**.
9. **Save:** Press **Enter** when **Yes** is displayed. Rename the file using a unique name and code.

5. Safety Instructions

1. **Sample Handling:** While the device is powered on, do not place samples directly into the thermal block wells; always use appropriate microcentrifuge tubes or vials in holders/disks.
2. **Contamination Control:** Designate separate, clean areas for “pre-amplification” and “post-amplification” work. Ideally, these should be in two different rooms to prevent cross-contamination.
3. **Ventilation:** Do not place any objects near the device that might obstruct the cooling fan or airflow.

6. Maintenance

1. **Documentation:** The user manual must be kept in an easily accessible location.
2. **Ventilation Clearance:** Due to the high ventilation requirements of the thermal cycler, a minimum clearance of **75 cm** must be maintained on both sides of the device.
3. **Power Protection:** Use a **stabilizer** or **UPS** to protect the device from electrical fluctuations.
4. **Cleaning Agents:** Do not use corrosive cleaners or abrasive/rough cloths for cleaning the device.
5. **Environment:** Store and operate the device in a dry environment.
6. **Fire Safety:** Do not place flammable materials near or on top of the thermal block.
7. **Consumables:** Use only standard microcentrifuge tubes that match the device’s required type, size, and volume.
8. **Shutdown:** Always press the **Off** button before disconnecting the device from the power supply.

⚠ IMPORTANT:

- If you are a beginner, you **must** notify a laboratory expert before proceeding.
- In the event of any technical malfunction or error, notify the laboratory expert immediately.

تاریخ اجرا:	تصویب کننده:	تأید کننده: دکتر محمد رضا عربستانی	تهیه کننده: حسین فضلی
تاریخ بازنگری:	سمت:	سمت: مدیر گروه میکروبیشناسی	سمت: سوپر وایزر آزمایشگاه
شماره صفحه: ۲ از ۱	تاریخ و امضاء:	تاریخ و امضاء:	تاریخ و امضاء: