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Eppendorf ThermoMixer® C – Features, Options, Applications

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Executive Summary

Primarily used in the field of life sciences, the Eppendorf ThermoMixer® C is a device for the temperature control and mixing of liquids or samples. The device offers a wide temperature control range (1 °C–100 °C), high flexibility with the use of diverse tube and plate formats and advanced mixing performance with the ²ᴰMix-Control technology and a mixing speed of 3000 rpm. With the new technology **condens.protect®**, the Eppendorf ThermoTop® prevents condensation on tube lids or plate sealing foils.

Overview

> **Exchangeable Eppendorf SmartBlocks™ (5 µL–50 mL)**
Use the convenient Eppendorf QuickRelease™ to quickly and easily replace SmartBlocks for frequently used tubes and plates.

> **²ᴰMix-Control**
The controlled and efficient mixing motion provides quick and thorough mixing of even the smallest volumes.

> **The menu**
Customize your Eppendorf ThermoMixer C to your needs: create, edit and save programs; activate the Interval Mix function; define ramp rates and select the required Time Mode in the menu. You can also change the program key assignment and device settings such as key lock, signal tones, contrast and languages in the menu.

> **The display**
All at a glance: temperature and mixing parameters, time, program name, program steps and active functions.

> **Eppendorf ThermoTop with condens.protect technology**
A heated lid is optionally available for Eppendorf SmartBlocks with 5 µL–2.0 mL tube and plate volumes: the Eppendorf ThermoTop prevents condensation droplets from forming on the tube inner wall and tube lid.

> **Program keys**
Quickly access your most frequently used mixing and temperature control conditions.

> **Short key**
For short mixing – the Eppendorf ThermoMixer C mixes at the selected speed when the short key is pressed.

> **Start/stop key and Pause**
Start and stop the temperature control and mixing processes, or activate a pause.

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Eppendorf SmartBlocks™
Eppendorf SmartBlock™ 0.5 mL
Eppendorf SmartBlock™ 1.5 mL
Eppendorf SmartBlock™ 2.0 mL
Eppendorf SmartBlock™ 5.0 mL
Eppendorf SmartBlock™ 15 mL
Eppendorf SmartBlock™ 50 mL
Eppendorf SmartBlock™ 12 mm
Eppendorf SmartBlock™ cryo thaw
Eppendorf SmartBlock™ plates
Eppendorf SmartBlock™ PCR 96
Eppendorf SmartBlock™ PCR 384
Eppendorf SmartBlock™ DWP 500
Eppendorf SmartBlock™ DWP 1000

Lid for Eppendorf SmartBlocks™
0.5 mL–2.0 mL, PCR 96 & 384, plates
> Lid (unheated Lid)
> Eppendorf ThermoTop® (heated Lid)

| *Technical data |
|---|
| > Max. 3000 rpm (in dependence of the SmartBlock) |
| > Regulated temperature: max. 15 °C below RT max. -100 °C |
| > Lowest selectable temperature value: 1 °C |
| > Temperature accuracy 20–45 °C: ± 0.5 °C; below 20 °C and above 45 °C: ±1 °C |
| > Heating speed: max. 6 °C/min |
| > Cooling speed: above RT: max. 2.5 °C/min; below RT: max. 0.8 °C/min |
| > Block homogeneity: 20–45 °C: ± 0.5 °C; < 20 °C or > 45 °C: ±1 °C |
| > Selectable Ramp rates |
| > Time Mode (Time/Temp Control) |
| > Interval Mix |
| > Short Mix |
| > 5 Program keys; 20 free definable program places |

*Complete technical data for each Eppendorf SmartBlock are available on www.eppendorf.com

The Eppendorf SmartBlocks

Various Eppendorf SmartBlocks are available in a volume range of 5 µL to 50 mL. The user-friendly Eppendorf QuickRelease technology makes replacing Eppendorf SmartBlocks easy and fast.
Please note!: The block will be initially placed at an angle against the rear edge of the universal mount and then engaged by applying pressure toward the rear.

Press the release head down to disengage the block so it can be removed.
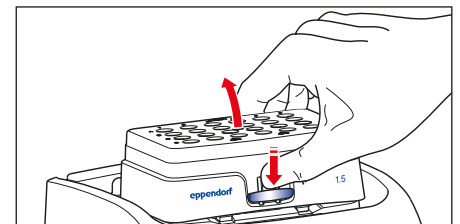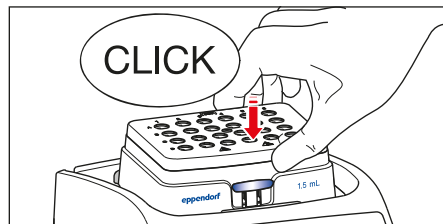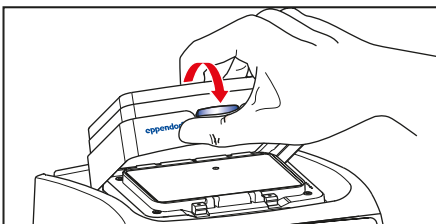
Figures 1 + 2: Attaching a thermoblock

Figure 3: Removing a thermoblock

The Eppendorf ThermoMixer C automatically detects the attached block; this ensures that the maximum mixing frequencies cannot be exceeded.
The best possible temperature accuracy can be guaranteed by individually adjusting an Eppendorf SmartBlock. Optimized temperature control for each block type keeps temperature overshoots and deviations caused by external interferences to a minimum.

To guarantee optimal performance over the entire period of use, Eppendorf offers a preventive maintenance program and certification services: your Eppendorf ThermoMixer C and Eppendorf SmartBlocks are regularly cleaned, checked and adjusted. Additional information is available at: www.eppendorf.com/epservices.

Eppendorf SmartBlocks for	Shape of boreholes			Eppendorf ThermoMixer C max rpm/max temp
	LxW (mm)	Depth (mm)	bottom shape	
microtest tubes 1.5 mL	Ø 11.0	34.7	conical	2000 rpm/100 °C
microtest tubes 2.0 mL	Ø 11.0	34.6	round	2000 rpm/100 °C
microtest tubes 0.5 mL	Ø 8.2	26.4	conical	2000 rpm/100 °C
vessel for 5.0 mL	Ø 17.4	53.0	conical	1000 rpm/100 °C
vessels with 11–12 mm diameter	Ø 12.1	34.5	conical 120°	2000 rpm/100 °C
96-well PCR plates, 0.2 mL PCR tubes	Ø 6.4	14.0	conical	2000 rpm/100 °C
384-well PCR plates	Ø 3.8	8.0	conical	3000 rpm/100 °C
MTP's & DWP's (SBS format)	130 x 88		flat	3000 rpm/100 °C
vessels for 15 mL/50 mL	Ø 17.4/29.8	106/102	conical	1000 rpm/100 °C
cryo tubes	Ø 12.7	31.7	flat	2000 rpm/100 °C

The display and its symbols

All important parameters are available at a glance using the clearly arranged digital display:

	Time, temperature, mixing speed	
	Program name	
	Program steps	
	Rotating arrows: Eppendorf **ThermoMixer C is active** – temperature control/mixing	
	Eppendorf **ThermoTop** – the symbol only appears in the display if the Eppendorf ThermoTop is attached and has been detected by the device.	
	Interval Mix – mixing phases and mixing pause are now activated in succession. The symbol appears if Interval Mix is activated and the mixing phases/mixing pauses have been defined: rotating arrows in the top left symbol represent the mixing phase; the vertical lines at the bottom right represent the mixing pause. Within programs, Interval Mix can only be selected using the menu.	
	Time Mode: Time Control	The symbols indicate which Time Mode has been selected using the menu. A more detailed description can be found in the "Selecting the Time Mode" section.
	Time Mode: Temp Control	
	Signal tones on	The menu is used to set the volume of signal tones or switch them off.
	Signal tones off	
	Key pad is open	When it is open, parameters can be changed during the run. The **key lock** function is selected using the menu.
	Key pad is locked	When the key pad is locked, parameters cannot be changed during the run. The **key lock** function is selected using the menu.

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^{2D}Mix-Control

The high mixing capacity of the ^{2D}Mix-Control technology, known from the MixMate®, combined with temperature control of samples, is available for the first time as the Eppendorf ThermoMixer C. ^{2D}Mix Control technology guarantees controlled and efficient mixing motion, regardless of tube geometry or sample properties. This enables the quick and complete mixing of the smallest

volumes and samples that are traditionally difficult to mix, for example, samples with high viscosity or salt concentrations. You achieve perfect mixing results even in very small wells of 384 plates. Anti-spill technology guarantees that samples will not spray and adjacent wells will not become contaminated, even at high mixing speeds.
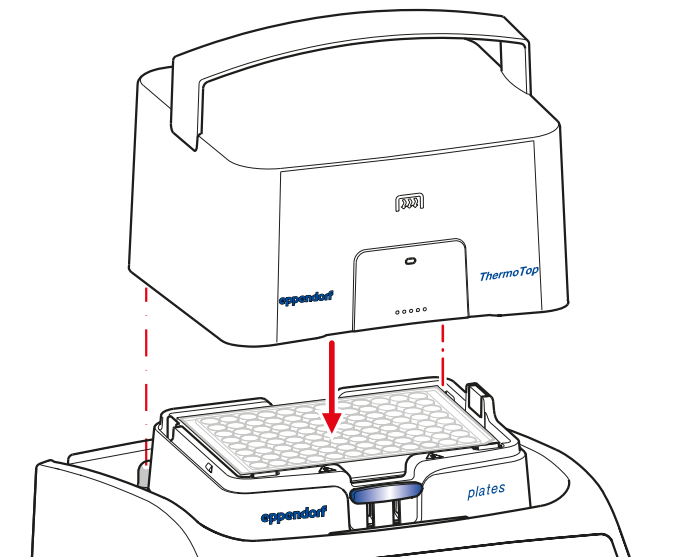
condens.protect® technology with Eppendorf ThermoTop

Optionally available for Eppendorf SmartBlocks 0.5/1.5 and 2.0 mL as well as Eppendorf SmartBlocks plates and PCR 96 & PCR 384 – labeled with the ***condens.****protect* symbol 〔♪♪♪〕 ® – is this heated lid. It prevents the formation of condensation.

! The Eppendorf ThermoTop should be placed upon the Eppendorf SmartBlock from the beginning of the temperature control. Thereby it is ensured that the Eppendorf ThermoTop has always an optimized temperature and thus, forming of condense water drops is prevented reliably from the beginning. Therefore using the Eppendorf ThermoTop causes a slight delay of the heat up speed of the Eppendorf SmartBlock.

! The temperature sensor of the Eppendorf SmartBlock reacts very sensitively to the sample temperature. Inserting cooler samples into a preheated Thermoblock can therefore lead to a momentarily descent of the displayed Eppendorf SmartBlock temperature. The same effect occurs if a cold Eppendorf ThermoTop is placed on a preheated Eppendorf SmartBlock.

When working with small volumes, preventing condensation on the lid and edge of the tube is especially important because a reduced sample volume can have undesirable effects on sensitive, biochemical reactions. With the Eppendorf ThermoTop and its ***condens.****protect* technology, centrifugation steps for "spinning down" drops on the tube lid or plate cover are a thing of the past.

The Eppendorf ThermoTop is placed on the device housing using the Eppendorf SmartBlock. If the lid is correctly attached, it will be automatically detected by the device. The LED on the lid will light up blue and the Eppendorf ThermoTop symbol appears in the display 〔▦〕. As soon as the temperature control process begins the blue LED on the lid will blink. If the temperature control has been completed, the lid symbol will light up blue again and the symbol disappears from the display after the Eppendorf ThermoTop has been removed.

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Start a simple mixing and temperature control procedure

Use the arrow keys to select the temperature, mixing speed and time. Press the **start/stop** key to start the run.
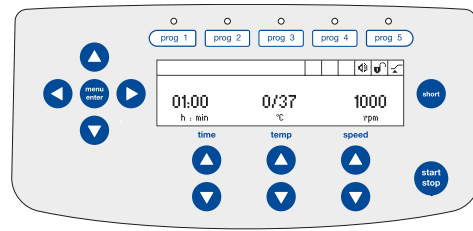
The program keys

You can use the 5 program keys (prog 1–prog 5) to quickly, directly access your most frequently used temperature control and mixing programs. The keys are factory preset but can be changed, renamed and saved to suit your needs.
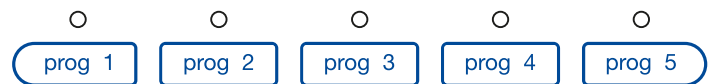
Select the program of your choice by pressing a **prog key**, and start it by pressing the **start/stop** key.

You can easily customize the displayed parameters to suit your needs, and immediately save the parameters by pressing the corresponding **prog key** for **at least 2 seconds**. These settings and renaming of the prog keys can also be done in the menu described as follows.
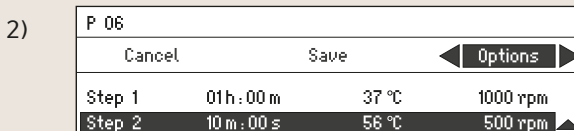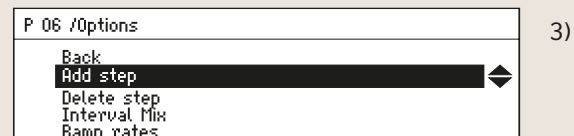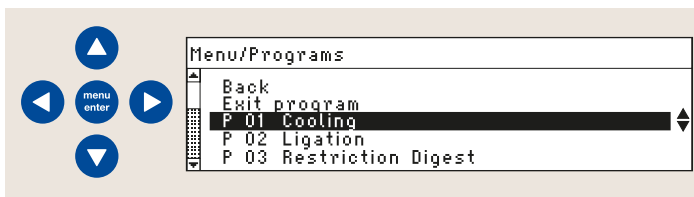
Program keys – factory settings
prog 1: Cooling: 8 °C; ∞; 0 rpm
prog 2: Ligation: 16 °C; 16 h, 0 rpm
prog 3: Restr. Digest: 37 °C; 1 h; 1000 rpm
prog 4: Prot. K Digest: 56 °C; 10 min; 1000 rpm
prog 5: Denaturation: 95 °C; 30 min; 0 rpm

Calling, creating, changing, naming and saving programs

With the Eppendorf ThermoMixer C, you can configure, name and save 20 programs with up to 4 steps per program. To access these functions, go to the menu and select

Programs. Here, you can decide if you would like to create a new program, load a program **(Load)** or edit a program **(Edit)**(1). Add or remove the program **Steps** under **Options** in the Edit mode. You can also activate the **Interval Mix** function and define ramp rates here (2; 3). Then you can name and **save** the program (4).

! At the end of the operating manual you can find a table where you might list your programs.

```
Menu/Programs
 Back
 Exit program
 P 01 Cooling
 P 02 Ligation
 P 03 Restriction Digest
```

1)
```
P 06 ELISA
 Back
 Load
 Edit
 Delete
```

2)
```
P 06
 Cancel        Save      ◄ Options ►

Step 1    01h:00m      37 ℃     1000 rpm
Step 2    10m:00s      56 ℃      500 rpm
```

3)
```
P 06 /Options
 Back
 Add step
 Delete step
 Interval Mix
 Ramp rates
```

4)
```
P 06
 Cancel        ◄     Save     ►

 A B C D E F G H I J K L M
 N O P Q R S T U V W X Y Z
 1 2 3 4 5 6 7 8 9 0 _ ←
```

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Options: Steps, Interval Mix and Ramp rates

1. Adding/removing steps
You can add up to 4 steps to each program, which enables the simple programming of more complex temperature control procedures. A practical cooling level can be defined as the final step in the program.

| For example: |
|---|
| Step 1: Denaturation |
| Step 2: Enzyme reaction |
| Step 3: Enzyme heat inactivation |
| Step 4: Cooling |

! A program starts always with step 1. It can't be started at another step. The active running step is signed with a star like this ☐ *Step 1 ☐. It is possible to see all program steps while another step is running. The shown step is signed then by a black background whereas the running step is displayed with the star. The example display above shows therefore the parameters of Step 2 but Step 1 is just running.

2. Interval Mix
This function can only be selected and activated within a program. This means that a program must be selected or created in order to use this function. To do so, go to the menu in the program mode, select or create a program and activate it in the Interval Mix options. There, you can define the duration of the mixing phase and mixing pause. Both phases are now alternately activated over the entire selected period.

! The settings of the Interval Mix function always refer to the step currently selected in a program and, therefore, must be separately defined for each desired step. The Interval Mix symbol then appears in the display for those steps where the Interval Mix is activated and defined.
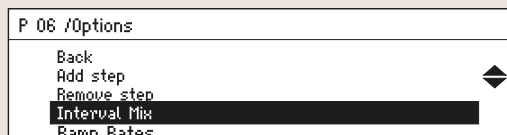
Examples of use

Nucleic acid extraction from agarose gels
The agarose gel is dissolved in high-salt buffer at 50 °C for 10 min. Interval Mix at 1400 rpm every 2 min for 15 s each improves the solubility of the gel slice.

> Eppendorf SmartBlock 1.5 mL
> Time Mode: Temp Control
> Pogram Step 1: temp: 55 °C; time: 16 h; speed: 800 rpm/
  Interval Mix: mixing pause 5 min/mixing time 1 min
> Program Step 2: temp: 7 °C; 0 rpm; time: ∞ (cooling)

Proteinase K digestion
Digestion of cell proteins – occasional mixing improves the lysis

> Eppendorf SmartBlock 1.5 mL
> Time Mode: Temp Control
> Pogram Step 1: temp: 55 °C; time: 16 h; speed: 800 rpm/
  Interval Mix: mixing pause 5 min/mixing time 1 min
> Program Step 2: temp: 7 °C; 0 rpm; time: ∞ (cooling)

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3. Ramp rates

Applications that require a controlled and reproducible heating or cooling speed can be executed using the Eppendorf ThermoMixer C. To do so, select the program in the menu and select the ramp rates in the options.

Select with help of the menu arrow keys between following speeds:

Cooling rates:
> max 1 °C/min
> max 0.5 °C/min
> max 0.1 °C/min
> maximal
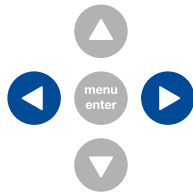
Heating rates:
> max. 3.0 °C/min
> max. 2.0 °C/min
> max. 1.0 °C/min
> max. 0.1 °C/min
> maximal

When the **maximum** option is selected, the device heats or cools at the maximum possible speed. This allows the device to reach values that can vary slightly based on the type of Eppendorf SmartBlock, load (total volume), initial and final temperature and ambient temperature.

Selecting the Time Mode (Time Control, Temp Control)

You can program the Eppendorf ThermoMixer C so that the device immediately starts with the time counting and mixing after you have pressed "start". Therefore, the time counting and the mixing process also start if the set temperature has not yet been reached – the device is in **Time Control mode**. However, with a relatively short temperature control period, the selected temperature may not be reached even though the programmed time has already elapsed. If this occurs, the **Temp Control mode** will provide a remedy: when the **Temp Control mode** is selected, the time counting and mixing process will only start after the selected temperature has been reached.

```
P 06 /Options/Ramp Rates
   Back
   Heating rate    ◄ max 1.0 °C/min ►
   Cooling rate       maximal
```
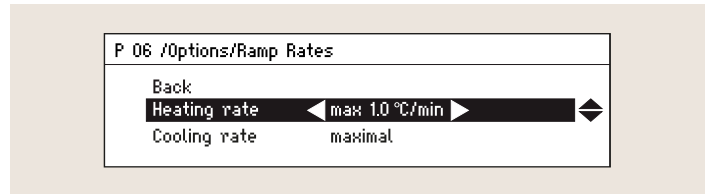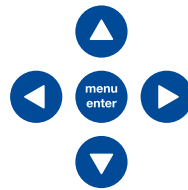
Examples of use

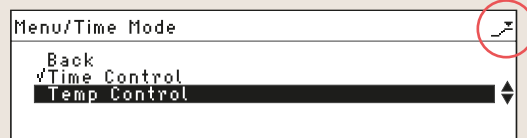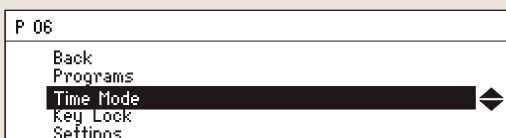**Specific annealing of oligonucleotide probes on DNA Polymorphy-Screening of pharmaceutical active agents**

For example
Controlled slow cooling down from e.g. 80 °C to 20 °C in e.g. 10 h (0.1 °C/min)
> Time Mode: Temp Control
> Heating rate: maximal
> Cooling rate: 0.1 °C/min
> Program Step 1: 80 °C; 300 rpm; time e.g. 30 min
> Program Step 2: 20 °C; 300 rpm; time e.g. 30 min
> Program Step 3: 80 °C; 300 rpm; time e.g. 30 min
> Program Step 4: 20 °C; 300 rpm; time: ∞
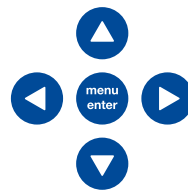
Time Mode	
Time Control	Temp Control
Time counting and mixing start immediately, regardless of whether the selected temperature has already been reached.	Time counting and mixing only start after the selected temperature has been reached.

! The **Temp Control mode** is always recommended when samples need to be incubated at a specific temperature for a specific period of time.

```
P 06
   Back
   Programs
   Time Mode
   Key Lock
   Settings
```

```
Menu/Time Mode
   Back
   √Time Control
   Temp Control
```

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The Pause function

The Pause function can be activated by pressing the **start/stop key for at least 2 seconds**. The time counting and mixing process are now interrupted, but the temperature will continue to be controlled. **Pause** appears in the display. The mixing process and time counting will be continued by pressing the **start/stop** key again.
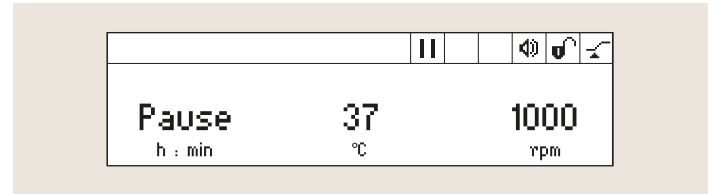
Example of use

! This function can be useful if the mixing process must be briefly interrupted to add enzymes, salts, catalysts, inhibitors or other materials to a sample during an assay. The time counting will be interrupted during the Pause. The temperature will continue to be precisely regulated.

Aborting a run:
if a program or a simple run is interrupted by briefly pressing the **start/stop** key, mixing starts if the key is pressed again. Time counting will start over from the very beginning.

! A pause between program steps must be programmed as a step.

Additional examples of use

The applications listed here serve as examples of how your device can be programmed. However, always observe the data in your protocol and modify the programming accordingly.

! The Eppendorf ThermoMixer C reliably regulates temperatures up to 15 °C below ambient temperature. That means e.g. 7 °C can be reached only when the ambient temperature in the lab does not exceed 22 °C.

Labeling of probes for hybridization experiments – e.g. random priming
> Eppendorf SmartBlock 1.5 mL or 0.5 mL
> Time Mode: Temp Control
> Program Step 1: 95 °C; 0 rpm; 3 min (denaturation); thereafter put the sample immediately on ice; addition of buffer, dNTP's, enzyme
> Program Step 2: 37 °C; 0 rpm; 2 h (incubation)
> Program Step 3: 70 °C; 0 rpm; 10 min (enzyme inactivation)
> Program Step 4: 7 °C: 0 rpm; time: ∞ (cooling)

Transformation of DH5α
> Eppendorf SmartBlock 1.5 mL
> Time Mode: Temp Control
> Program Step 1: 42 °C; 60 sec, without shaking! (heatshock); put the sample immediately on ice
> Program Step 2: 37 °C; 300 rpm; 30 min–2 h (Transformation of DH5α; slightly mixing increases the transformation efficiency)

Restriction digest of DNA
> Eppendorf SmartBlock 1.5 mL
> Time Mode: Temp Control
> Program Step 1: 37 °C; 500 rpm; 1 h/Interval Mix: mixing pause 5 min/mixing time 1 min
> Program Step 2: 65 °C; 500 rpm; 10 min (enzyme inactivation)
> Program Step 3: 7 °C: 0 rpm; time: ∞ (cooling)

DNAse I digest of RNA preparations
> Eppendorf SmartBlock 1.5 mL
> Time Mode: Temp Control
> 37 °C; 800 rpm; 1 h (DNAse I digestion)
> 65 °C; 800 rpm; exakt 5 min (heat inactivation of the DNAse)

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Reverse transcription
> Eppendorf SmartBlock 1.5 mL
> Time Mode: Temp Control
> Program Step 1: 70 °C; 800 rpm;
  10 min (denaturation of RNA and primers)
> Program Step 2: 37–42 °C; 800 rpm;
  1 h (first strand cDNA-synthesis with oligo-(dT) primers)
> Program Step 3: 70 °C; 800 rpm; 30 min (inactivation of
  the reverse transcriptase by heat inactivation)
> Program Step 4: 7 °C: 0 rpm; time: ∞ (cooling)

Phosphorylation of linker-DNA
> Eppendorf SmartBlock 1.5 mL
> Time Mode: Temp Control
> Program Step 1: 37 °C; 800 rpm;
  30 min–1 h (incubation step)
> Program Step 2: 65 °C; 800 rpm;
  20 min (stop of the reaction by heat inactivation)
> Program Step 3: 7 °C: 0 rpm; time: ∞ (cooling)

Dephosphorylation of linear DNA molecules
> Eppendorf SmartBlock 1.5 mL
> Time Mode: Temp Control
> Program Step 1: 37 °C; 800 rpm;
  1 h (incubation step)
> Program Step 2: 75 °C; 800 rpm; 10 min (stop of the
  reaction by heat inactivation) – (or in order to stop the
  reaction add 5 mM EDTA pH 8.0)
> Program Step 3: 7 °C: 0 rpm; time: ∞ (cooling)

Growth of bacteria
In Deepwell plates (DWP) or 2 mL microreaction tubes
> Eppendorf SmartBlock plates or
  Eppendorf SmartBlock 2.0 mL
> Time Mode: Temp Control
> Program Step 1: 37 °C; 800 rpm; 20–24 h (has to be
  tested in accordance to the bacteria density)
> Program Step 2: 7 °C; 500 rpm; time: ∞ (cooling)

In 15 mL or 50 mL Falcon® tubes
> Eppendorf SmartBlock 15 mL or
  Eppendorf SmartBlock 50 mL
> Time Mode: Temp Control
> Program Step 1: 37 °C; 800 rpm; 20–24 h (has to be
  tested in accordance to the bacteria density)
> Program Step 2: 7 °C; 500 rpm; time: ∞ (cooling)

**Immunoprecipitation with Antibody-Sepharose
(covalently bound)**
> Eppendorf SmartBlock 1.5 mL
> Time Mode: Temp Control
> Program Step 1: 21 °C; e.g. 800 rpm;
  2 h (or 4 °C; 500 rpm; 18 h (over night))
> Program Step 2: 4 °C; 0 rpm; time: ∞ (cooling)

Immunoprecipitation of the Antigen
> 4 °C; 1000 rpm or higher in order to suspend the
  Sepharose; 1,5–3 h

Dissoziation of Ag and Ak-Sepharose
> 100 °C; 5 min

Immunoprecipitation-Recapture
> Eppendorf SmartBlock 1.5 mL
> Time Mode: Temp Control
> Program Step 1: 21 °C; 5 min; e.g. 500 rpm
  (incubation with elution buffer)
> Program Step 2: 95 °C; 5 min; e.g. 500 rpm
  (incubation with elution buffer)
> Program Step 3: 21 °C; 10 min; e.g. 500 rpm
  (incubation with lysis buffer)
> Program Step 4: 4 °C; 0 rpm; time: ∞ (cooling)