

Missing a Second Incubation Temperature?

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Executive Summary

Many standard methods in the molecular biology workflow require more than one temperature for successful completion. Popular examples are heat inactivation of enzymes, denaturation of DNA, RNA Proteins, cDNA synthesis, labelling of probes, or heat shock transformation of DNA into bacteria. For an optimized and time-saving process, a second incubation device is needed, resulting in investment and additional

lab space. The SmartExtender is an easy-to-use, effective accessory for the current Eppendorf ThermoMixer® C and Fx, as well as the ThermoStat® C. It offers parallel incubation of samples at a second, independent temperature. This increases the convenience, extends the capacity, broadens the applicational range, decreases contamination risks, and saves lab space compared to a second device.

Introduction

Procedures in molecular biology labs often require quick incubation steps for thawing of frozen buffers, pre-warming and pre-incubation of buffers and samples. Frequently, more than one incubation temperature is required for a successful protocol.

For instance, a typical protocol for cDNA first strand synthesis describes incubation temperatures such as 65 °C for the initial denaturation step, 50 °C for the synthesis, and 85 °C for termination of the reaction. Common ThermoBlocks on a ThermoStat or Eppendorf ThermoMixer require some time to heat up or cool down to the next needed incubation temperature. To solve the challenge, you can reserve one of your valuable PCR Cyclers for these applications or use a second ThermoStat/Eppendorf ThermoMixer.

Another example is the transformation of DH5α: the initial heat shock is usually done in a water bath at 42 °C for 1 min. The specific transformation step of the plasmid together with the cells follows at 37 °C for at least 30 min or up to 2 h, depending on the protocol. Using a water bath for heat shock requires additional space for the device and, moreover, bears a contamination risk because of impure or contaminated water. Another possibility is the usage of a second ThermoStat or Eppendorf ThermoMixer.

There is a definite need for improvement. A convenient and cost-efficient set-up is described here by using just one Eppendorf ThermoMixer.

Solution & Benefits

A solution for this challenge is the SmartExtender (Figure 1). This accessory offers a comfortable incubation tool which can easily be used as an add-on to the existing Eppendorf ThermoMixer C/Fx, as well as ThermoStat C*.

The accessory extends the capacity with up to 12 x 1.5 mL microtest tubes for higher throughput and increases the flexibility of the device. The SmartExtender provides an independent heating function for a second incubation temperature that can either be used in parallel to an Eppendorf SmartBlock™ or separately for quick incubations. This can include, for example, thawing of buffers, pre-incubations, denaturation, or termination reactions. By using an additional heating element on the Eppendorf

ThermoMixer/ThermoStat, the temperature control is independent from the SmartBlock (second temperature) for a second application. All SmartBlocks are usable in parallel to the SmartExtender, ensuring their full functionality of heating, cooling, and mixing, and providing the high flexibility you are used to. The SmartExtender can actively heat from 3 °C above room temperature up to 110 °C to cover all common applications. This significantly extends the range of applications within the Eppendorf ThermoMixer Family.

The applications listed in Table 1 below are examples of how the SmartExtender can be used.

*Firmware-update required, self-extracting file available at www.eppendorf.com/ThermoMixer

Table 1: SmartExtender applications

Method & Incubation temperatures	SmartExtender: SE or SmartBlock: SB	Comment & benefit
Superscript® III First-Strand Synthesis SuperMix¹		
1. 65 °C; 5 min (denaturation primer & RNA)	SE	The SmartBlock 1.5 mL heats up to 50 °C for the synthesis. Denaturation of primer & RNA can be done in parallel on the SmartExtender. No second device is necessary.
2. 50 °C, 50 min (synthesis)	SB	
3. 85 °C, 5 min (termination)	SE	Immediate termination without delay. No need of a second device or even a PCR cyclor.
Transformation of DH5α		
1. 42 °C, 60 seconds (heat shock)	SE	No use of a water bath necessary. Avoids contamination risks.
2. 37 °C, 30 min – 2h (transformation)	SB	
Restriction digest of DNA		
1. 37 °C, 1 h	SB	Immediate enzyme inactivation possible without delay and without a second device.
2. 65 °C, 10 min (enzyme inactivation)	SE	
Labeling of probes for hybridization experiments – e.g. random priming		
1. 95 °C, 3 min	SE	All 3 incubation steps can be done with a single Eppendorf ThermoMixer. Denaturation and termination reaction can be done in the SmartExtender. No delays emerge from heat up or cooling down times of the SmartBlock.
2. 37 °C, 2 h (incubation)	SB	
3. 70 °C, 10 min (enzyme inactivation)	SE	

Further Application Examples

- > Thawing of frozen reagents
- > Pre-warming of buffers, samples
- > Denaturation of DNA, RNA, proteins: e.g. 95 °C
- > Sample preparation for denaturing gel electrophoresis: 95 °C
- > Dehydration of protein spots on preparative gels: 30 °C, 5 min
- > Trypsin digestion of proteins from gel pieces: 37 °C, 8 h



Figure 1
A: A 1.5 mL SmartExtender filled with tubes
B: The 1.5 mL SmartExtender installed on an Eppendorf ThermoMixer

Summary

Many standard methods in the molecular biology workflow require more than one temperature for successful completion: quick incubation steps for thawing of frozen buffers, pre-warming steps, and pre-incubation of buffers and samples, as well as sequential temperature incubations in a series or workflow. The Eppendorf SmartExtender is the perfect tool to support this. The user gets the benefit of increased convenience, saves money and lab space, and has the opportunity to exchange incubation in a water bath, thus minimizing contamination risks.

[1] Superscript® III First-Strand Synthesis SuperMix, Product Information Sheet, Thermo Fisher Scientific

About Eppendorf

Eppendorf is a leading life science company that develops and sells instruments, consumables, and services for liquid-, sample-, and cell handling in laboratories worldwide. Its product range includes pipettes and automated pipetting systems, dispensers, centrifuges, mixers, spectrometers, and DNA amplification equipment as well as ultra-low temperature freezers, fermentors, bioreactors, CO₂ incubators, shakers, and cell manipulation systems. Associated consumables like pipette tips, test tubes, microtiter plates, and disposable bioreactors complement the instruments for highest quality workflow solutions.

Eppendorf was founded in Hamburg, Germany in 1945 and has more than 3,000 employees worldwide. The company has subsidiaries in 25 countries and is represented in all other markets by distributors.

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