



Applied Biosystems StepOnePlus Real-Time PCR Machine

1. Introduction

A StepOnePlus real-time PCR machine located at the main laboratory of IBEC at the Hèlix building is available for use by all IBEC researchers.



The StepOnePlus System from Applied Biosystems supports any real-time PCR application, such as:

- SNP genotyping
- Gene expression profiling
- MicroRNA expression
- Translocation analysis
- Gene detection
- Viral load analysis

Furthermore, the software for StepOnePlus system supports a variety of analysis methods, including:

- Absolute quantitation
- Standard curve
- Relative quantitation
- Relative standard curve
- Comparative CT ($\Delta\Delta$ CT)
- Presence/absence assays with an internal positive control
- Melt curve analysis
- Genotyping (including real-time amplification)

To find out more information about the StepOnePlus real-time PCR please visit the Applied Biosystems website at <u>www.appliedbiosystems.com</u>.

2. User Pre-requisites

- <u>Only IBEC researchers can use the StepOnePlus real-time PCR machine.</u>
- Before using the StepOnePlus real-time PCR system you must obtain an individual account on the StepOnePlus computer. To obtain it, please contact the Core Facilities staff at corefacilities@ibecbarcelona.eu.





• With your personal user name and password you can access the computer locally to run the instrument or from your personal computer to retrieve your data.

You must not disclose your password to any person who is not authorized to use the instrument.

3. Reservations

- To access the instrument, you have to book it previously using the online booking application.
- If you are 30 minutes late after the start of the booking-time the whole reservation is considered cancelled and the equipment is available to everybody.
- Appointments that cannot be held must be cancelled as early as possible in order to free up the slots for other users.

4. Usage Rules

- Wear powder-free gloves and change them frequently to help prevent contamination of the sample with epithelial cells from the hands.
- Prepare samples away from the real-time PCR machine.
- Use an appropriate tray support for plate or tubes. Do not allow the bottom of the plate or tubes to become dirty. Fluids and other contaminants that adhere to the bottom of the plates or tubes can contaminate the sample block and cause an abnormally high background signal.
- Once finished your experiment, leave the area around the machine cleaned, the real-time PCR machine empty and logged off the computer.





IMPORTANT: Plates and tubes must be compatible with the machine. The SetpOnePlus machine must be used with the following consumables, all from Applied Biosystems:

	Catalog number
MicroAmp® Fast Optical 96-well reaction plate with barcode (0.1	4346906
ml)	
MicroAmp® Optical Adhesive Film - PCR Compatible (100	4311971
films)	
MicroAmp® Optical Adhesive Film (25 films)	4360954
MicroAmp® Fast 8-Tube Strip (0.1 ml)	4358293
MicroAmp® Fast Reaction Tube with cal (0.1 ml)	4358297
MicroAmp® Optical 8-Cap Strip	4323032

5. Data storage and retrieval

- Your files must be saved to the location indicated to you after your training session:
 - 1. Click on "My computer", then on "AB SW&DATA (D:)".
 - 2. Access the "sharing" directory to enter your personal folder.

In order to make the access to your personal folder easier you can create a shortcut of it on your desktop.

- Notice that files stored in other areas are subjected to possible deletion.
- The use of a USB to retrieve your data is not recommended, instead you can access to the computer using the local area network to do so:
 - 1. Use the following IP address on the Internet explorer: 10.6.46.45
 - 2. Access the directory "sharing".
 - 3. Enter your personal folder, using your username and password.

6. General

• If you have any problems with the real-time PCR machine, please report them to the Core Facilities Unit at <u>corefacilities@ibecbarcelona.eu</u>.