PCR Assistant

Software manual
from software version 40.1
Table of contents

1 Operating instructions .............................................. 5
  1.1 Using this manual ........................................... 5
  1.2 Symbols used .............................................. 5

2 Product description .................................................... 7
  2.1 Software description ......................................... 7
    2.1.1 Special features of the PCR Assistant on the epMotion 5070. .............................................. 7

3 Operation ............................................................... 9
  3.1 Preparing applications ........................................ 9
    3.1.1 Updating the labware library ........................... 9
    3.1.2 Preparing tubes and adapters ......................... 9
    3.1.3 Filling the tubes ..................................... 10
  3.2 Using the assistant ............................................ 11
    3.2.1 Starting the assistant ................................. 11
    3.2.2 Entering information ................................ 12
    3.2.3 Ending the assistant .................................. 12
  3.3 Creating an application ...................................... 13
    3.3.1 Selecting labware and pipette tips .................. 13
    3.3.2 Compose Mastermix assistant ......................... 17
    3.3.3 Normalization assistant ............................... 20
    3.3.4 Dilution Series assistant ............................. 22
    3.3.5 Setup Reactions assistant ............................ 25
  3.4 Equipping the worktable ..................................... 28
  3.5 Starting the application .................................... 28

4 Displaying, saving and printing protocols ...................... 31

5 Troubleshooting ....................................................... 33
  5.1 Error messages ............................................... 33

6 Ordering information ................................................ 35
  6.1 Dispensing tools ............................................. 35
  6.2 Pipette tips .................................................. 35
  6.3 Consumables ................................................. 35
1 Operating instructions
1.1 Using this manual

Your epMotion operating manual consists of hardware instructions and software instructions. Short instructions are available for optional software enhancements.

The operating manual is part of the product.

The current version of the operating manual can be found on our webpage: www.eppendorf.com.

- Read the operating manual in full before using the device.
- Store the operating manual at an easily accessible location.
- The device may only be transferred with the operating manual.
- If the operating manual is lost, replace it immediately. Please contact Eppendorf AG for further details.

1.2 Symbols used

<table>
<thead>
<tr>
<th>Depiction</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Actions in the specified order</td>
</tr>
<tr>
<td>2.</td>
<td>Actions without a specified order</td>
</tr>
<tr>
<td>▷</td>
<td>List</td>
</tr>
<tr>
<td>Text</td>
<td>Display text or software text</td>
</tr>
<tr>
<td>▶️</td>
<td>Additional information</td>
</tr>
</tbody>
</table>
2 Product description
2.1 Software description

The PCR Assistant offers step-by-step operating sequences for special applications. You can use the PCR Assistant without having any experience in programming applications.

For the PCR Assistant, you need the dispensing tools TS 50 and TS 300.

The PCR Assistant consists of 4 assistants. You can use them to realize the following PCR operating sequences:

PCR Assistant Compose Mastermix - Creating a mastermix
• Create PCR mastermixes from ready mastermixes or from single components like buffers, polymerases, dNTPs, primers, probes. The PCR Assistant determines which volume is required for each component.

PCR Assistant Normalization - Normalizing concentrations
• Dilute the DNA/RNA samples in order to obtain identical concentrations of each sample. You can enter the concentrations manually or import them from a file.

PCR Assistant Dilution Series - Creating dilution series
• Dilute the DNA/RNA standards in series in order to obtain calibration curves for quantitative PCR.

PCR Assistant Setup Reactions - Creating reactions
• Create complete reactions by combining samples with mastermixes. Create replicates of a reaction.

2.1.1 Special features of the PCR Assistant on the epMotion 5070

If you use the Assistant for the epMotion 5070, samples and diluent must be in a labware.
3 Operation

3.1 Preparing applications

3.1.1 Updating the labware library

You can combine a wide variety of plates, tubes and racks and insert them into the epMotion.

Update the labware library as follows:

1. Check if the labware definition and labware combination is available in the labware library.
2. If the labware definition is not available in the labware library, import the labware definition.
3. If the labware combination is not available, create the labware combination, e.g., tubes and racks.
4. In order to receive a clearly arranged selection, deactivate any labware which is not required.

For information on how to receive labware definitions and on how to work with labware definitions, refer to the software operating manual.

3.1.2 Preparing tubes and adapters

Prepare the tubes and plates as follows:

1. Open the tubes.
2. Insert the tubes into the rack so that the lids do not hide the tube openings.
3. Place PCR plates without full edge into a PCR 96 thermoblock.

Observe the filling volume of the tubes.
If the required volume exceeds the allowable filling volume, your application will not start.
3.1.2.1 Equipping the PCR 96 thermoblock with PCR tubes

If you work with PCR tubes with hinged lids, equip the PCR 96 thermoblock as follows:

1. Place the PCR tubes into the positions of the thermoblock column by column, beginning with column 1.
2. Keep every 2nd column free.

3.1.3 Filling the tubes

- Position the samples in the source labware row by row.
  - For racks, begin with position 1.
  - For plates, begin with position A1.
3.2 Using the assistant
3.2.1 Starting the assistant

1. Switch on the epMotion.
   The epBlue start screen appears.

2. Select an application in the Assistant area. Click on the application symbol.
   The application will open; the start screen appears.
   All applications consist of several program steps. Each program step will be shown in a window. All windows have the same appearance.

![Assistant start screen](image)

**Fig. 3-2: Assistant start screen**

1. **File menu**
   Information on the File menu can be found in the software operating manual.

2. **Status area**
   epMotion status

3. **Work area**
   Information on the current program step

4. **Information area**
   Access to all program steps. When you click on a program step, it will be shown in the work area.

5. **Navigation area**
   
   - `< button - return to the last step.
   - `> button - go to the next step.

6. **Cancel button**
   End the assistant and return to the start screen.
3.2.2 Entering information

For information on using the software, refer to the software operating manual.

Automatically show the screen key pad.
- epBlue automatically shows a key pad if you have selected an input field.

Manually show the screen key pad.
- In the File menu, select the Show keyboard entry.

Checking the entries
- The software checks each entry. If an entry leads to a conflict, the input field is outlined in red. Information about the conflict will appear below the input field.

Entering tube positions
- The positions of a rack are numbered by row. The top left position has the number 1. Enter the tube position in a rack as a number.
- The rows of a plate are designated by letters, the column by numbers. In order to specify the position of a well, enter the row and the column, e.g. A1.

3.2.3 Ending the assistant

1. Click on the Cancel button to end the assistant.
   The entered values will not be saved.
2. Alternatively, in the File menu, you can click on the Exit to Start Screen entry.
3.3 Creating an application
3.3.1 Selecting labware and pipette tips
3.3.1.1 Selecting source labware for samples

At the beginning of an application, you select the labware. The assistant will display the labware available in the labware library. The *Labware information* field shows the description of the labware.

![Select Labware for samples window](image)

*Fig. 3-3: Select Labware for samples window*

In order to select any labware, proceed as follows:

**Prerequisites**
- The *Select labware for samples (source 1)* window is opened.

1. In order to check the filling level of the labware with the optical sensor, activate the *Volume detection* checkbox.
   - A checkmark will appear in the checkbox.
   - If the optical sensor does not check the level of the tubes, enter the labware volume manually after starting the application.
   - The optical sensor does not check the level of plates. Enter the wells’ volume after starting the application.

2. Select labware from the appropriate folder.
   - Information on the selected labware is displayed in the *Labware information* column.
Special features of the *Setup Reactions* assistant

![Diagram of Select labware for samples window in the Setup Reactions application](image)

Fig. 3-4: *Select labware for samples* window in the *Setup Reactions* application

**Pipette button**
- Pipetting samples

**Multidispense button**
- Dispensing samples

3. In order to pipette samples, select the *Pipette* button.
4. In order to dispense samples, select the *Multidispense* button.
   - The selected button will be highlighted in blue.
5. Press the *Next* button.
3.3.1.2 Selecting the source labware for diluent

In order to select any labware, proceed as follows:

Prerequisites

- The Select labware for diluent (source 2) window is opened.

1. In order to select labware for diluent, select labware from the appropriate folder. Information on the selected labware is displayed in the Labware information column.

2. In order to place samples and diluent in the same labware, activate the Place diluent in the sample labware (source 1) as well checkbox.

   A checkmark will appear in this checkbox. For the diluent, the labware selected in step 1 will be used. The settings of the optical sensor are transferred from step 1. The Volume detection checkbox is not active.
3.3.1.3 Selecting the destination labware

For selecting the destination labware, proceed as follows:

Prerequisites
- The Select labware for destination window is opened.
- In order to use the source labware as destination labware as well, activate the Place ... in the source labware as well checkbox.
  - This option is not available for all PCR assistants.
- Select the labware, (see Selecting source labware for samples on p. 13).

When you have equipped every second column of the thermoblock with PCR tubes, use the labware definition Thermoblock with Plates > EP_Tube_Thermo_0_2_48.

3.3.1.4 Selecting pipette tips

You require 50 μL pipette tips and 300 μL pipette tips. For pipetting steps with volumes ≤ 50 μL, use the dispensing tool TS 50. For pipetting steps with volumes > 50 μL, use the dispensing tool TS 300.

You can use pipette tips with or without filters. Proceed as follows:

Prerequisites
- The Type of pipette tips window is opened.

1. In order to use pipette tips with filters, activate the Use filter tips checkbox.
2. In order to use pipette tips without filters, deactivate the Use filter tips checkbox.
3.3.2 **Compose Mastermix assistant**

Prerequisites

- The labware and the pipette tips have been selected (see p. 13).
- The *Type of pipette tips* window is opened.

1. Press the Next button.
   - The *Define required mastermix volume* window will appear.

![Fig. 3-6: Define required mastermix volume window](image)

**Total number of reactions** input field

Number of reactions per mastermix

**Total volume (sample + mastermix) per reaction** input field

Total volume per PCR reaction.

**Sample (template) volume per reaction** input field

Sample volume per PCR reaction.

**Excess volume per mastermix** input field

Additional mastermix volume, e.g., for compensating the dead volume of the destination location.

The assistant uses these entries to calculate the required mastermix volume. If you create multiple mastermixes, the entries will be used for all mastermixes.

2. Fill in the input fields.
3. Press the Next button.
   - The *Define name and positions of mastermix volume* window will appear.

4. Define the name and the position of the mastermix in the destination labware. Create one row in the table for each mastermix to be created.
   - Entering the name is optional.
5. Press the *Next* button.
   
   The *Define mastermix components* window will appear.

![Define mastermix components window](image)

**Fig. 3-7:** *Define mastermix components* window

**Mastermix 1 tab**
Each mastermix is represented in a tab. Each component is represented in a row.

**Add button**
Adding a table row

**Edit button**
Editing a table row

**Delete button**
Deleting a table row

**Pdf Preview button**
Displaying a mastermix as PDF file

6. In order to edit a component of the mastermix, press on the corresponding row.
   The row is highlighted in blue.

7. Press the *Edit* button.
   An input mask will appear for the mastermix component.
Fig. 3-8: Define mastermix components input mask

**Component name input field**
Name of the component

**Position in source input field**
Position of the component in the source labware

**Concentration button**
Define the component by entering the initial and the final concentration.

**Volume button**
Define the component by entering the volume per reaction.

**Stock concentration input field**
Initial concentration of the component

**Final concentration input field**
Final concentration of the component in the PCR reaction

**Volume/reaction input field**
Volume of the component for one reaction

**Volume/mastermix field**
Calculated mastermix volume

8. In order to save entries, press the **Save** button.
   The input mask will be closed.

9. Fill in the input fields for all components.
10. Define the components for all mastermixes.
11. Press the **Next** button.
   The **Overview worktable** window will appear (see p. 28).
3.3.3 **Normalization assistant**

**Prerequisites**
- The labware and the pipette tips have been selected (see p. 13).
- The *Type of pipette tips* window is selected.

1. Press the *Next* button.
   The *Define concentrations and labware positions* window will appear.

   ![Fig. 3-9: Define concentrations and labware positions window](image)

   **Final concentration for all samples input field**
   Final concentration of all samples

   **Position of diluent in ... input field**
   Position of the diluent in the source labware

   **Fixed final volume button**
   Execute the normalization with defined final volume for dilutions. Vary sample volumes and diluent volumes.

   **Fixed sample volume button**
   Realize normalization with fixed sample volume. Vary diluent volume and final volume.

2. Fill in the input fields.
3. Create a table row for each sample.
4. In order to edit a sample, mark the table row and press the *Edit* button.
   An input mask will appear.
5. Fill in the input fields
6. In order to save the entries, press the **Save** button. The input mask will be closed.
7. Edit all samples.

**CSV import**

8. Alternatively, create a sample table with a CSV file. To this purpose, load the CSV file with the **Load input File** button.

The CSV file must have the following column headings:

<table>
<thead>
<tr>
<th>Position</th>
<th>Concentration</th>
<th>Destination Position</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>99.5</td>
<td>A1</td>
<td>sample 1</td>
</tr>
<tr>
<td>2</td>
<td>75.2</td>
<td>A2</td>
<td>sample 2</td>
</tr>
<tr>
<td>3</td>
<td>74.8</td>
<td>A3</td>
<td>sample 3</td>
</tr>
<tr>
<td>4</td>
<td>86</td>
<td>A4</td>
<td>sample 4</td>
</tr>
<tr>
<td>5</td>
<td>91.6</td>
<td>A5</td>
<td>sample 5</td>
</tr>
<tr>
<td>6</td>
<td>72.6</td>
<td>A6</td>
<td>sample 6</td>
</tr>
<tr>
<td>7</td>
<td>79.7</td>
<td>A7</td>
<td>sample 7</td>
</tr>
<tr>
<td>8</td>
<td>85.1</td>
<td>A8</td>
<td>sample 8</td>
</tr>
<tr>
<td>9</td>
<td>69</td>
<td>A9</td>
<td>sample 9</td>
</tr>
<tr>
<td>10</td>
<td>78</td>
<td>A10</td>
<td>sample 10</td>
</tr>
</tbody>
</table>
9. Press the **Next** button.
   The **Overview worktable** window will appear (see p. 28).

### 3.3.4 **Dilution Series assistant**

**Prerequisites**
- The labware and the pipette tips have been selected (see p. 13).
- The **Type of pipette tips** window is selected.

1. Press the **Next** button.
   The **Define dilution parameters** window will appear.
Diluent position in ... input field
Position of the diluent in the source labware

Arrange diluted samples by column button
Arrange dilution series in the destination labware by column

Arrange diluted samples by row button
Arrange the dilution series in the destination labware by row

Dilution factor 1/x input field
Dilution factor

Number of dilution steps input field
Number of dilution steps

2. Fill in the input fields.

The dilution series has to fit in one row or one column of the destination labware.
Example: In a rack with 24 positions (4 rows and 6 columns), a dilution series is arranged by row. The first dilution step is performed in the tubes in the 2nd column. A maximum of 5 dilution steps is possible.

3. Press the Next button.
The Define labware positions window will appear.

Fig. 3-14: Define labware positions window

Table
Each table row represents one dilution series. The table shows the source position of the sample and the first and the last dilution position.
4. Create one table row for each dilution series. To this purpose, press the Add button.

5. In order to edit a dilution series, mark the table row and press the Edit button.
   For each dilution series, an input mask will appear.

   ![Define labware positions input mask window](image)

   **Source position input field**
   Source position of the undiluted sample

   **First step input field**
   Position of the 1st dilution step.

   **Last step field**
   Calculated position of the last dilution step.

6. Fill in the input fields.
7. Click on the Save button.
   The input mask will be closed.
8. Edit all dilution series.
9. Press the Next button.
   The Overview worktable window will appear (see p. 28).
3.3.5  **Setup Reactions assistant**

Prerequisites

- The labware and the pipette tips have been selected (see p. 13).
- The *Type of pipette tips* window is selected.

1. **Press the Next button.**
   
The *Define Mastermixes* window will appear.

   ![Define Mastermixes window](image)

   **Fig. 3-16: Define Mastermixes window**

   **Position in ... column**  **Name column**
   
   Position of the mastermix in the source labware  Name of the mastermix, optional

2. **Fill in the input fields.**

3. **Press the Next button.**
   
The *Define reaction volume* window will appear.
4. Fill in the input fields.
5. Press the Next button.

The Define number of reactions window will appear.
6. Fill in the input fields.
7. Press the Next button.
   The Arrangement in destination window will appear.

![Arrangement in destination window](image)

**Fig. 3-19:** Arrangement in destination window

- **Number of samples (templates) input field**
  Number of samples including standards and checks

- **Number of replicate reactions per sample input field**
  Number of reaction batches per sample (replicates)

- **Arrangement in destination buttons**
  Arrangement of samples, mastermixes and replicates in the destination labware

- **Center destination checkbox**
  Arrange the samples in the center of the destination labware

8. In order to export the pattern of the destination labware as CSV file, press the Export as CSV button.
9. In order to export the pattern of the destination labware as PDF file, press the Export as PDF button.
10. Press the Next button.
    The Overview worktable window will appear (see p. 28).
3.4 Equipping the worktable

Fig. 3-20: Window: Worktable overview

1. Equip the epMotion worktable corresponding to the figure.
2. Position the TS 50 dispensing tool at location T1. Position the TS 300 dispensing tool at location T2.
3. Empty the waste box.

3.5 Starting the application

If the entries have been completed, start the application. Proceed as follows:

Prerequisites
- The Overview worktable window is open.

1. Click on the Save button to save the application under a new name.
   Saved applications can be opened and changed in the Application Editor. A description of this procedure can be found in the software operating manual.
2. Click on the Run button to start the application.
   The Application Runner starts.
3. Press the Next button.
   The application is loaded.
4. Set the parameters for the UV lamp and HEPA air filter if applicable.
   For information on setting the UV lamp and HEPA air filter, refer to the software operating manual.
5. Press the Next button.
6. Set the functions of the optical sensor.

For information on using the optical sensor, refer to the software operating manual.

If you activate the Detect volumes radio button, the level detection will be switched on for this run. The optical sensor realizes the level detection for the labware for which you activated the Volume detection in labware checkbox (Fig. 3-3 on p. 13).

If you activate the Input volumes manually radio button, enter liquid volumes manually.

7. Click on the Run button to start the application.

For information on controlling an application, refer to the software operating manual.

8. When the application is finished, press on the Exit to Start Screen button.
4 Displaying, saving and printing protocols

The software automatically saves the last executed application of each assistant. The existing application will be overwritten when a new application is started.

For information on using protocols, refer to the software operating manual.
5 Troubleshooting
5.1 Error messages

Information on error messages can be found in the software operating manual and the epMotion hardware operating manual.

If an error occurs, check the following items first:

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>An error message appears before the start of the application.</td>
<td>• A volume in the application is larger than the filling volume of the selected vessel.</td>
<td>▸ Select a vessel that holds this volume.</td>
</tr>
<tr>
<td>Your labware does not appear in the selection.</td>
<td>• The labware library does not have a definition of this labware.</td>
<td>▸ Import the labware definition into the labware library.</td>
</tr>
<tr>
<td></td>
<td>• The labware was deactivated in the labware library.</td>
<td>▸ Activate the labware in the labware library.</td>
</tr>
<tr>
<td>The optical sensor does not detect the level.</td>
<td>• There is foam is on the liquid.</td>
<td>▸ Briefly centrifuge vessels.</td>
</tr>
<tr>
<td></td>
<td>• The surface of the liquid is uneven, e.g., due to the meniscus of the liquid or the formation of foam</td>
<td>▸ Then quickly vortex or shake the vessels.</td>
</tr>
<tr>
<td>The optical sensor does not detect the level.</td>
<td>• There is not enough liquid in the vessel. The detection limit of the optical sensor has not been reached.</td>
<td>▸ Enter the volume manually.</td>
</tr>
</tbody>
</table>
6 Ordering information

For comprehensive ordering information on pipette tips, labware and accessories, please refer to the hardware operating manual.

6.1 Dispensing tools

<table>
<thead>
<tr>
<th>Order no. (International)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5280 000.010</td>
<td>Single-channel dispensing tool TS 50</td>
</tr>
<tr>
<td></td>
<td>Volume range 1 μL - 50 μl</td>
</tr>
<tr>
<td>5280 000.037</td>
<td>Single-channel dispensing tool TS 300</td>
</tr>
<tr>
<td></td>
<td>Volume range 20 μ - 300 μl</td>
</tr>
<tr>
<td>5280 000.053</td>
<td>Single-channel dispensing tool TS 1000</td>
</tr>
<tr>
<td></td>
<td>Volume range 40 μL - 1000 μl</td>
</tr>
</tbody>
</table>

6.2 Pipette tips

<table>
<thead>
<tr>
<th>Order no. (International)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0030 014.413</td>
<td>epT.I.P.S. Motion Filter 50 μL</td>
</tr>
<tr>
<td></td>
<td>10 racks with 96 tips each</td>
</tr>
<tr>
<td></td>
<td>PCR clean</td>
</tr>
<tr>
<td>0030 014.456</td>
<td>epT.I.P.S. Motion Filter 300 μL</td>
</tr>
<tr>
<td></td>
<td>10 racks with 96 tips each</td>
</tr>
<tr>
<td></td>
<td>PCR clean</td>
</tr>
</tbody>
</table>

6.3 Consumables

<table>
<thead>
<tr>
<th>Order no. (International)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0030 123.301</td>
<td>Eppendorf Safe-Lock Tube 0.5 mL</td>
</tr>
<tr>
<td></td>
<td>500 pieces, clear</td>
</tr>
<tr>
<td></td>
<td>PCR clean</td>
</tr>
<tr>
<td>0030 123.328</td>
<td>Eppendorf Safe-Lock Tube 1.5 mL</td>
</tr>
<tr>
<td></td>
<td>1,000 pieces, colorless</td>
</tr>
<tr>
<td></td>
<td>PCR clean</td>
</tr>
<tr>
<td>0030 123.344</td>
<td>Eppendorf Safe-Lock Tube 2.0 mL</td>
</tr>
<tr>
<td></td>
<td>1,000 pieces, colorless</td>
</tr>
<tr>
<td></td>
<td>PCR clean</td>
</tr>
<tr>
<td>0030 128.648</td>
<td>twin.tec PCR Plate 96, skirted</td>
</tr>
<tr>
<td></td>
<td>low profile, wells colorless, 25 pieces</td>
</tr>
<tr>
<td></td>
<td>clear</td>
</tr>
<tr>
<td>0030 128.575</td>
<td>twin.tec PCR Plate 96, semi-skirted</td>
</tr>
<tr>
<td></td>
<td>Wells colorless, 25 pieces</td>
</tr>
<tr>
<td></td>
<td>standard profile, clear</td>
</tr>
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<td>0030 128.575</td>
<td>twin.tec PCR Plate 96, semi-skirted</td>
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<tr>
<td></td>
<td>Wells colorless, 25 pieces</td>
</tr>
<tr>
<td></td>
<td>standard profile, clear</td>
</tr>
<tr>
<td>Order no. (International)</td>
<td>Description</td>
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<tr>
<td>---------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>0030 133.307</td>
<td>twin.tec PCR Plate 96 unskirted</td>
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<td></td>
<td>Wells colorless, 20 pieces</td>
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<tr>
<td></td>
<td>low profile, clear</td>
</tr>
<tr>
<td>0030 133.366</td>
<td>twin.tec real-time PCR Plate 96 skirted</td>
</tr>
<tr>
<td></td>
<td>wells white, 25 pieces</td>
</tr>
<tr>
<td></td>
<td>white</td>
</tr>
<tr>
<td>0030 132.513</td>
<td>twin.tec real-time PCR Plate 96 semi-skirted</td>
</tr>
<tr>
<td></td>
<td>wells white, 25 pieces</td>
</tr>
<tr>
<td></td>
<td>white</td>
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<tr>
<td>0030 132.700</td>
<td>twin.tec real-time PCR Plate 96 unskirted</td>
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<td>wells white, 20 pieces</td>
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<td>low profile, white</td>
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<tr>
<td>0030 124.332</td>
<td>PCR Tubes 0,2 mL</td>
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<tr>
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<td>1,000 pieces</td>
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<td>PCR clean, colorless</td>
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<tr>
<td>0030 124.820</td>
<td>PCR Tube Strips + Cap Strips</td>
</tr>
<tr>
<td></td>
<td>flat, 10 × 12 strips</td>
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<tr>
<td>0030 127.811</td>
<td>PCR Film</td>
</tr>
<tr>
<td></td>
<td>adhesive, 100 pieces</td>
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<tr>
<td>0030 127.820</td>
<td>PCR Foil</td>
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<td>0030 132.904</td>
<td>Masterclear real-time PCR Film</td>
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<tr>
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<td>adhesive, 100 pieces</td>
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