Multiplate® analyzer
Quick Start Guide
Document information

<table>
<thead>
<tr>
<th>Manual version</th>
<th>Software version</th>
<th>Revision date</th>
<th>Change description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>2.04</td>
<td>May 2013</td>
<td>Adapted to software version 2.04.</td>
</tr>
</tbody>
</table>

Revision history

Edition notice

This information is intended for operators of the Multiplate analyzer. It provides basic information to start working with the analyzer.

Every effort has been made to ensure that all the information is correct at the time of publishing. However, Roche reserves the right to make any changes necessary without notice as part of ongoing product development.

For more comprehensive information, refer to the Multiplate analyzer Operator’s Manual.
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Getting started

The Multiplate software uses the Windows 7 operating system. A password is required in order to start (Windows-Login):

user name  multiplate

password  multiplate

The software then starts up automatically and begins heating the measurement block up to 37 °C. The current temperature is displayed in the status bar. The instrument will reach the specified temperature range within 10 minutes.
Roche recommends performing an electronic control measurement before the first measurement of a shift (Multiplate > Start electronic QC). The system must run for at least 20 minutes before starting the electronic control measurement. Prepare the reagents. Fill the pre-heating tubes with diluent solution (0.9% NaCl; NaCl/CaCl₂ when using citrated blood), place into position, and prepare the tips. Before using the electronic pipette inspect the filter and replace it if necessary.

Prepare your test cells (check expiry date and the magnetic stirrer inside), and store the collected blood at room temperature (e.g. in the reagent holder). Samples should be analyzed within the period of 0.5–3 hours after blood collection. Never put the sample tubes on a rotating mixer.
**WARNING**

Infection by samples and associated materials
Contact with samples containing material of human origin may result in infection. All materials and mechanical components associated with samples of human origin are potentially biohazardous.

- Exercise the normal precautions required for handling all laboratory reagents and patient samples. Disposal of all waste material should be in accordance with local guidelines.

**Related topics**

- For information on how to change the pipette filter, see *Maintenance of the electronic pipette* (p. 35)
Reagent handling

▶ To prepare reagents

1. Carefully reconstitute the contents of one vial of the reagent by adding 1.0 mL of distilled or deionized water.

2. Gently swirl reagent and allow vial to stand closed for 10 minutes at 18-25 °C.

3. Swirl the vial carefully to produce a homogeneous solution before use. Avoid the formation of foam.
To aliquot into single day portions

1. To achieve maximum stability after reconstitution, pipette $\geq 100$ μL aliquots of the reagent into aliquot vials for daily use.

2. Store the aliquots appropriately.
   
   Aliquot vials must be closed between measurements to avoid evaporation as this will create concentration fluctuations. For this reason the manufacturer does not recommend aliquots of less than 100 μL.

3. Once the reagent is divided into the aliquot vials close the safety cap tightly. The following pages describe storage conditions.

💡 The reagent vials should be marked with the date of reconstitution. Do not refreeze reagents. Original vials of reconstituted reagent should be refrigerated unless in use, and stored as per manufacturers instructions.
Reagent storage conditions

For lot-specific information always refer to the current package insert.

Unopened reagent vials

Store unopened reagent vials at 2–8 °C. The lyophilized reagents are stable up to the stated expiration date.
Reconstituted reagents

ADPtest, RISTOtest, TRAPtest, ASA Reagent, Prostaglandin E1 Reagent

Once reconstituted the reagents are stable for 7 days at 2–8 °C (in the original reagent vial).

Storage at < -20 °C increases stability to 4 weeks. After one time thawing the reagent is stable for 24 hours at 18–25 °C.
**Reagent storage conditions**

**ASPltest**

Once reconstituted the reagent is stable for 24 hours at 2–8 °C.

Storage at < -20 °C increases stability to 4 weeks. After one time thawing the reagent is stable for 24 hours at 18–25 °C.

**COLtest**

Once reconstituted the reagent is stable for 7 days at 2–8 °C (in the original reagent vial).

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_Do not freeze the COLtest reagent!_
Handling of pre-heating tubes

Fill the tube with diluent solution (0.9% NaCl; NaCl/CaCl₂ when using citrated blood) at the beginning of a Multiplate session and place it into one of the pre-heating positions on the analyzer.
The tubes are also directly available from Sarstedt:
Part no. 60.550.100 (www.sarstedt.com).

**WARNING**

**Erroneous results due to inappropriate pre-heating tubes**

Using inappropriate pre-heating tubes can lead to erroneous test results.

- Pre-heating tubes (REF 06675654001) must be used, otherwise the correct temperature of diluent solution cannot be guaranteed.

**WARNING**

**Contamination of NaCl (and presence of preservatives)**

Contamination of NaCl (and presence of preservatives) could influence aggregation results.

- Tubes should be replaced regularly and filled with sterile saline solution (0.9% NaCl).
Performing tests using the electronic pipette

1. Choose the **Auto Pipette** button.

2. Enter the patient ID and select the desired test.

   Up to 5 tests can be run simultaneously. User defined tests are marked with [ud] in the list of tests.
3 Choose the **Check and start measurement series** button.

4 Place test cells into the measuring positions that are listed on the screen.

5 Connect the sensor cable(s) to the test cell(s).
6 Choose the **Start pipetting program** button.

Instructions are shown on the screen.

7 Press the blue start button on the electronic pipette to execute each pipetting step.

An arrow indicates the current pipette step. Subsequent steps are marked with a dot, executed steps with a tick.
Performing tests using the electronic pipette

8 Pipette the diluent solution and the blood sample into the test cells.

   The 3-minute incubation time starts automatically.

   An acoustic signal sounds at the end of each incubation period and the operator is asked to add the appropriate reagent to the test cells immediately.

9 Add the appropriate reagent to the test cells.

   Measurement starts automatically when the last pipetting step of each channel is completed.
10 In the channel display the status icons show the progress:

- Running
- Completed: The measurement is complete and the data are stored.

11 After the tests are complete, print the results, if required.

- Choose the **F6: Print** button.
- Select the channels whose results you want to print.
- Choose the **Print** button. When printing is complete, the icon on the left is displayed.
Performing tests using the electronic pipette

12 Remove the test cells and discard them according to the local regulatory standards.

13 Clear channels.
   - Choose the F7: Clear channel button.
   - Select the channels you want to clear.
   - Choose the Clear button.
Electronic pipette: avoiding handling errors

Continuously pressing the Start button (blue) during communication between the pipette and the analyzer may interfere with the communication, therefore it is necessary to wait until the next instruction is displayed on the screen before proceeding. Always follow the instructions displayed on screen.

The last step must be completed and the next pipette instruction must be displayed on the screen before using the eject function. Care and patience are required when handling the electronic pipette.

⚠️ Do not press any key on the pipette during initialization of the electronic pipette.
Add the reagent (e.g. ASPltest, TRAPtest) deep into the test cell.

Execute all of the pipetting steps sequentially, according to the instructions on the screen.

A visual inspection of the aspirated volume in the pipette tip is recommended before adding the solution into the test cell.

This avoids operating errors due to aspirated air or other malfunctions.

The images illustrate the accurate pipette tip fill levels for the volumes 20 μL and 300 μL.
Avoiding operator errors

Store blood samples at room temperature (18–25 °C), do not heat the sample.

Never put the sample tubes on a rotating mixer.

Do not remove the magnetic stirrer! Ensure that a stirrer is present in the test cell.

Diluent solution (0.9% NaCl; NaCl/CaCl₂ when using citrated blood) must be pre-heated for a minimum of 10 minutes before use.
Avoiding operator errors

Place the test cell firmly into the measuring position.

After manually pipetting the reagent it is important to start the test immediately. Pipette the reagent deep into the test cell.
Performing tests using a manual pipette

- **To perform tests using a manual pipette**
  1. Place the test cell(s) into the measuring position(s).
  2. Connect the sensor cable(s), ensure that the plug is firmly connected.
Performing tests using a manual pipette

3 Perform the pipetting. Pipette:

- 300 μL 0.9% NaCl + 300 μL hirudinized/heparinized blood
or
- 300 μL 0.9% NaCl/CaCl₂ + 300 μL citrated blood

4 Start the timer for incubation (warming and equilibration): Choose the F2: Start timer button.

5 The 3-minute countdown is displayed in the status bar of the main window.

When the standard 3-minute incubation time has elapsed, an acoustic signal is sounded.

💡 Adding the reagent and starting the test must be performed simultaneously and separately for each channel.
6 After incubation, pipette the reagent deep into the test cell.

7 Choose the **F3: Start test** button or press <F3>.

8 Select the channel (using the mouse or the keys 1-5) and confirm with **Start** or **Enter**.

Analysis begins for the selected channel.

9 Choose the **F4: Enter test/ID** button to enter a patient ID and select a test name for every started channel.

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**Performing tests using a manual pipette**
Performing tests using a manual pipette

10 Observe the status icons.
   This icon is displayed when the measurement is complete and the data are stored.

11 When the tests are complete, print the results, if required.
   • Choose the F6: Print button.
   • Select the channels whose results you want to print.
   • Choose the Print button. When printing is complete, the icon on the left is displayed.

12 Remove the test cells and discard them according to the local regulatory standards.

13 Clear channels.
   • Choose the F7: Clear channels button.
   • Select the channels you want to clear.
   • Choose the Clear button.

Related topics
• For a summary of the test procedures, see Test procedures for hirudinized/heparinized blood (p. 29) and Test procedures for citrated blood (p. 30).
Test procedures for hirudinized/heparinized blood

1. Pipette
   300 μL saline 0.9% (37 ºC) + 300 μL hirudinized/heparinized blood

2. Incubation
   3 minutes

3. Pipette
   20 μL TRAPtest
   20 μL ADPtest
   20 μL ASPtest
   20 μL COLtest
   12 μL RISTOlow
   50 μL RISTOhigh

   3a. Pipette
      20 μL Prostaglandin E1

   3b. Pipette
      20 μL ADPtest

4. Measuring
   6 minutes

For ADPtest HS (high sensitivity)
### Test procedures for citrated blood

1. **Pipette**
   - 300 μL saline 0.9%/3mM CaCl₂ (37 °C) + 300 μL citrated blood

2. **Incubation**
   - 3 minutes

3. **Pipette**
   - 20 μL TRAPtest
   - 20 μL ADPtest
   - 20 μL COLtest

4. **Measuring**
   - 6 minutes

1. **Pipette**
   - 300 μL saline 0.9% (37 °C) + 300 μL citrated blood

2. **Incubation**
   - 3 minutes

3. **Pipette**
   - 20 μL ASPtest
   - 12 μL RISTOlow
   - 50 μL RISTOhigh

4. **Measuring**
   - 6 minutes
The Multiplate analyzer continuously records platelet aggregation. The adhesion and aggregation of platelets onto the Multiplate sensors generate an increase in impedance which is transformed into arbitrary aggregation units (AU) and plotted against time.

The most important parameter is the area under the aggregation curve (AUC). It is affected by the total height of the aggregation curve as well as by its slope and is best suited to express the overall platelet activity. Two AUC units are used: U and AU*min. The first unit (U) is the preferred one.

Two more parameters are calculated: The aggregation (Agg) is the increase of impedance during analysis. The velocity (Vel) is the maximum slope of the aggregation curve.
Two curves are assessed using the two independent sensors in the Multiplate test cell. The parameters calculated by the software are the mean values of the data of each curve.

Above the curve the AUC bars with reference and target ranges are displayed. Within the AUC bars the black bars (A) indicate the actually measured AUC result. The green zones (B) represent the reference and the target range. They can be individually defined for each test.

Press Ctrl+K or choose **Switch curve mode** from the **Measurements** menu to toggle between the overlapped curves (representing the duplicate measurements) or the filled curve (reflecting the mean value of the measurements).
## Sensitivity of reagents

<table>
<thead>
<tr>
<th>Test</th>
<th>Reagent (Final concentration)</th>
<th>ASA</th>
<th>Clopidogrel(^{(a)})</th>
<th>GPIIb/IIIa antagonists</th>
<th>vWS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAPtest</td>
<td>TRAP-6 (32 μM)</td>
<td>-</td>
<td>-/+(b)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ASPItest</td>
<td>ArA (0.5 mM)</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>COLtest</td>
<td>Collagen (equates to an activity of 3.2 μg/mL)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ADPtest</td>
<td>ADP (6.5 μM)</td>
<td>+</td>
<td>+(c)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ADPtest HS(^{(d)})</td>
<td>PGE1 (equates to an activity of 9.4 nM) ADP (6.3 μM)</td>
<td>-</td>
<td>+(e)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>RISTOhigh</td>
<td>Ristocetin (0.77 mg/mL)</td>
<td>-/+</td>
<td>-/+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

\(\text{Reagent sensitivity} \)

(a) and other ADP-receptor inhibitors (prasugrel, ticagrelor, ticlopidine)

(b) some samples show a lower TRAPtest on clopidogrel therapy

(c) The ADPtest is used by most centers for monitoring of clopidogrel and other ADP-receptor blockers using citrated or hirudinized blood.

(d) HS = high sensitivity

(e) The ADPtest HS is recommended when using heparinized blood to monitor clopidogrel and other ADP-receptor blockers.
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAP-6</td>
<td>Thrombin receptor activating peptide-6</td>
</tr>
<tr>
<td>ArA</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>PGE1</td>
<td>Prostaglandin E1</td>
</tr>
<tr>
<td>ASA</td>
<td>Acetylsalicylic acid</td>
</tr>
</tbody>
</table>
Maintenance of the electronic pipette

To perform electronic pipette maintenance

1. The electronic pipette filter should be inspected frequently.
   Cleanliness helps to prevent clots forming on the filter and maintain accurate tip fill levels.

2. Use REF 06675620001 filters as supplied by Roche.
3 Grip the filter using the forceps and pull it out of the pipette.

4 Using the forceps pick up a new filter and insert it into the pipette cone. Push it firmly against the stop position.

💡 Make sure not to soil the new filter while you install it.