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Quick Reference Guide: Calculating Proton Efflux Rate (PER) Data Using Agilent Seahorse Wave Desktop and Agilent Seahorse Wave Pro Software

Introduction

The Agilent Seahorse XF pH sensor probe measures changes in the concentration of free protons in the microchamber in real-time; this data is referred to as extracellular acidification rate (ECAR). ECAR can be transformed into a rate that reflects the number of protons extruded over time, proton efflux rate (PER). Table 1 shows the types of acidification data.

Table 1. Comparison of metrics of extracellular acidification.

Type of Acidification Data	ECAR	PER
Detects extracellular acidification from all sources?	Yes	Yes
Changes with media formulation?	Yes	No
Accounts for buffer factor of the system?	No	Yes
How is buffering accounted for?	None	Buffer factor
Displayed while running assay?	Yes	No

Proton efflux rate

PER is a rate measurement calculated in Wave Pro software and Wave Desktop software. PER delivers a quantitative measure of extracellular acidification and is only available in postrun assay results. It is not displayed while running an assay; ECAR is the only real-time acidification data reported during an XF assay. The three rate options displayed in the **Rate Measurement** drop-down menu are: OCR, ECAR, and PER. PER is calculated using the following equation:

PER (pmol H+/min) = ECAR (mpH/min) × BF (mmol/L/pH) × Geometric Volume (µL) × Kvol

Table 2. Wave volume variables.

Variable	Unit	Description
Extracellular Acidification Rate (ECAR)	mpH/min	Rate data measured during an assay, reported as the rate of change in mpH in an assay well.
Buffer Factor (BF)	mmol/L/pH	Measure of the *in situ* buffer capacity obtained in XF instruments, and accounts for buffer capacity of both medium and sensor system.
Geometric volume	µL	Physical volume of the measurement microchamber assuming its completely sealed.
Volume scaling factor (Kvol)	No units	Volume scaling factor used to account for total proton production in the measurement chamber.

Display PER data in Wave

Wave Pro software calculates and reports total PER on the Overview, OCR versus ECAR, and Data analysis views for the XF Pro and XF HS Mini Analyzers. Wave Desktop software calculates and reports total PER on the Overview, OCR versus ECAR, and Data analysis views for the XFe96, XFe24, XFp, and XF96. Use the **Rate Measurement** drop-down menu and select **PER** to display on the kinetic graph (Overview analysis view), or scatter plot (OCR versus ECAR analysis view). To correctly calculate and display PER, the assay media and background well buffer factor must be entered in Wave Pro software and Wave Desktop software.

Requirements to display PER data in Wave

To correctly calculate and display PER, the buffer factor of the system (and all assay wells) must be known.

Wave will automatically display PER if the following conditions are true:

1. The Sensor Cartridge is valid (not expired).

2. The assay media does not have a valid buffer factor value assigned, or is not one of the following assay media catalog options in Wave software:

   – Glycolytic Rate Assay Medium (DMEM-based)

   – Glycolytic Rate Assay Medium (RPMI-based)

   – Seahorse XF DMEM Medium, pH 7.4

   – Seahorse XF RPMI Medium, pH 7.4

3. The assay was run using a supported plate type and cartridge.

   – The following plate types are **Not Supported**:

     - Spheroid

     - Islet

     - V28

4. All background wells have a valid buffer factor value.

If PER is NOT displayed

If you are using an assay medium other than XF Glycolytic Rate Assay Medium (DMEM-based or RPMI-based), then Buffer Factor must be determined manually and entered per the procedure **Display PER for assays run with custom assay media** on page 5.

• Refer to the *Agilent Seahorse XF Buffer Factor Protocol* (available in the XF Glycolytic Rate Assay Report Generator download folder and XF Real-Time ATP Rate Assay Report Generator download folder) for guidance

If you used the above medium but did not set up the media details in the assay template, follow the procedure below:

Figure 1. Assay Media option.

1. Open the assay result file in Wave software.

2. Click **Modify**.

3. Click **Add** next to **Assay Media**. (See Figure 1.)

4. To select the assay medium, use the **Media** catalog drop-down menu. (See Figure 2.)

   – **Seahorse XF DMEM Medium, pH 7.4**

   – **Seahorse XF RPMI Medium, pH 7.4**

   – **Glycolytic Rate Assay Medium (DMEM-based)**

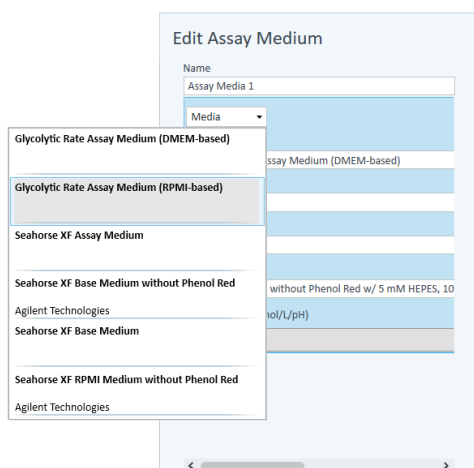   – **Glycolytic Rate Assay Medium (RPMI-based)**

Figure 2. Assay medium media menu.

5. To configure Background Well Buffer Factor, click **Assay Media**. (See Figure 3.)

6. Click **Configure**.

7. Under the column header **Default Buffer Factor**, check the boxes next to each background well, and click **Save**. (See Figure 3.)
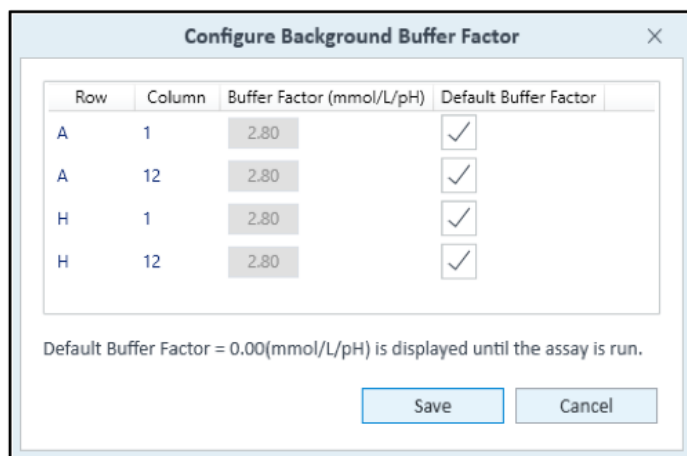

Figure 3. Configure background buffer factor.

8. Assign the **Assay Media** to each group in the group list. First click **Collapse/Expand All** to display the Group Definitions for each group.

9. Use the **Media** drop-down menu add the assay media to each group *Seahorse XF DMEM or RPMI Medium, pH 7.4 Glycolytic Rate Assay Medium (DMEM-based) or (RPMI-based)*
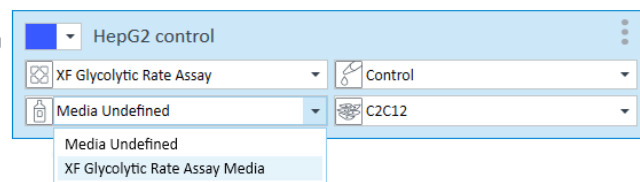

Figure 4. Media drop-down menu.

10. When finished, click **Apply**.

11. The kinetic graph and scatter plot will display PER data when selected using the **Rate** drop-down menu.

12. When finished with analysis, save the changes made to the result file.

Display PER for assays run with custom assay media

Prior to running the assay, calculate the Buffer Factor of the custom assay medium. Follow the steps in the *Agilent Seahorse XF Buffer Factor Protocol* (available in the XF Glycolytic Rate Assay Report Generator download folder and the XF Real-Time ATP Rate Assay Report Generator download folder) for guidance.

1. Open the assay result file in **Wave** software.

2. Click **Modify**.

3. Click **Add** next to **Assay Media**.

4. In the Edit Assay Medium window, enter the **Buffer Factor** in the field provided.

5. Next, click **Assay Media** again to configure Background Well Buffer Factor.

6. Click **Configure**.

7. Under **Buffer Factor (mmol/pH)**, type in the same Buffer Factor value and click **Save**. (See Figure 5.)
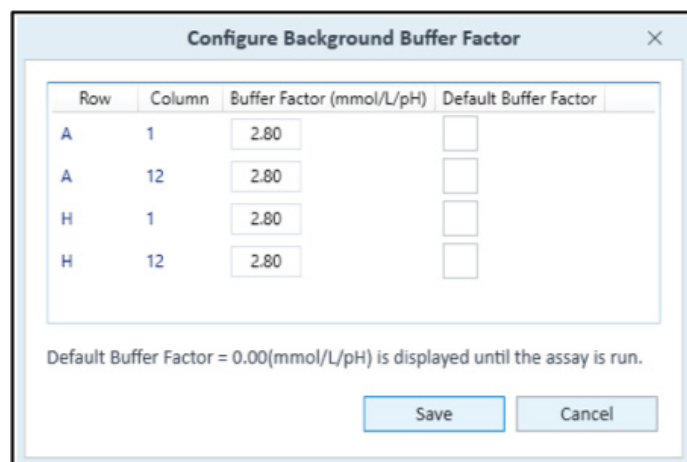

Figure 5. Type in the same buffer factor value.

3

8. Assign the **Assay Media** to each group in the group list. First click **Collapse/Expand All** to display the Group Definitions for each group.

9. To select the appropriate medium for each group, use the **Media** drop-down menu.

10. When finished, click Apply.

11. The kinetic graph and scatter plot will display PER data when selected using the **Rate** drop-down menu.

12. When finished with analysis, save the changes made to the result file.

Export total PER data

Export total PER data

PER data is included in both Microsoft Excel and GraphPad Prism file exports. (See Figure 7.) For manual PER data analysis or graphing purposes, export data from Wave to either file type using the **Export** function located in the top-level ribbon.
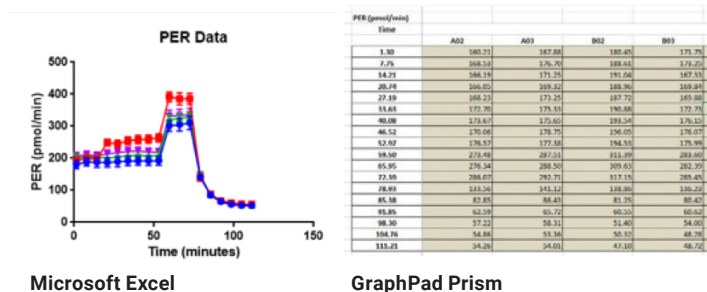

Microsoft Excel   **GraphPad Prism**

Figure 7. Exported data examples.

Helpful links

Learn about the capabilities of Wave Pro software and download the latest version: https://www.agilent.com/en/product/cell-analysis/real-time-cell-metabolic-analysis/xf-software/seahorse-wave-pro-software-2007523

Learn about the capabilities of Wave software and download the latest version: www.agilent.com/en-us/products/cell-analysis-(seahorse)/seahorse-wave-software

Learn more about options for measuring glycolysis: www.agilent.com/chem/glycolysisXF

Keep in touch with the latest news about Seahorse XF technology: www.agilent.com/chem/discoverXF

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