

Quick Reference Guide: Calculating Proton Efflux Rate (PER) Data



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 Production Rate (PPR) is the original metric for this rate; Agilent has recently introduced a new and improved metric, Proton Efflux Rate (PER), which better accounts for the buffering factor and effective volume of the transient microchamber. Table 1 compares these measurements of extracellular acidification.

Table 1. Comparison of metrics of extracellular acidification.

Type of acidification data:	ECAR	PPR	PER
Unit of measure	mpH min	pmol H+ min	pmol H+ min
Detects extracellular acidification from all sources?	Yes	Yes	Yes
Changes with media formulation?	Yes	No	No
Accounts for buffer factor of the complete system?	No	No	Yes
System buffering parameter used?	None	Buffer Capacity	Buffer Factor
Accounts for effective volume of the transient microchamber?	No	No	Yes
Displayed while running assay?	Yes	No	No

Proton Efflux Rate

PER is a new rate measurement calculated in Wave software (version 2.4 and higher). PER delivers a more accurate measure of extracellular acidification and is only available in post-run assay results. It is not displayed while running an assay. ECAR is the default reported acidification data in Wave software. The three rate options displayed in the **Rate Measurement** drop-down menu are: OCR, ECAR, and PER (previously PPR was the default). PER is calculated using the following equation:

$$\text{PER (pmol H}^+\text{/min)} = \text{ECAR (mpH/min)} \times \text{BF (mmol/L/pH)} \times \text{Geometric Volume (\mu L)} \times \text{Kvol}$$

Table 2. PER-related variables in Wave 2.4

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 <i>in situ</i> buffer capacity obtained in XF instruments, and accounts for buffer capacity of both medium and sensor system. For standard XF Glycolytic Rate Assay media, this value is prepopulated.
Geometric volume	μL	Physical volume of the measurement micro-chamber assuming its completely sealed.
Volume scaling factor (Kvol)	No units	Empirically-derived scaling factor used to account for total proton production in the measurement chamber.

Display PER data in Wave

Wave software calculates and reports total PER on the Overview, OCR vs. ECAR, and Data analysis views for the following XF Analyzers: XFe96, XFe24, XFp, and XF96. Use the **Rate Measurement** drop-down menu to select **PER** as the rate to display on the kinetic graph (Overview analysis view), or scatter plot (OCR vs. ECAR analysis view).

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:

- The Buffer Factor was automatically read from the sensor cartridge
 - If you are attempting to analyze data from an older run, the buffer factor might not be encoded in the barcode label
- The assay template was designed with media = XF Glycolytic Rate Assay Medium (DMEM-based or RPMI-based) in all Groups

- The assay was run using a supported plate type & cartridge

- The following are Not Supported:
 - XF24 and XF24-3 sensor cartridges
 - Spheroid, Islet, and V28 plate types

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) for guidance and an assay template to calculate Buffer Factor

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

- Open the assay result file in Wave software.
- Click **Modify**.
- Click **Add** next to **Assay Media**. (See Figure 1.)
- 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)**

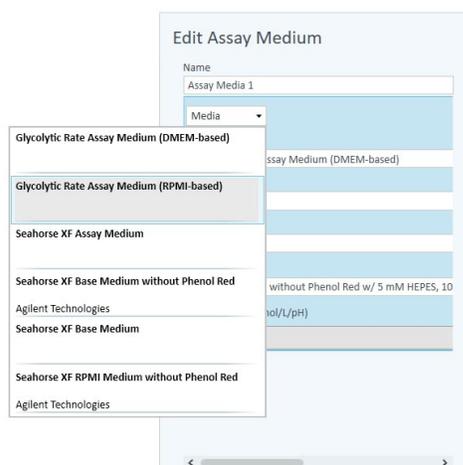


Figure 2. Assay medium media menu

- To configure Background Well Buffer Factor, click **Assay Media**. (See Figure 3.)
- Click **Configure**.
- Under the column header **Default Buffer Factor**, check the boxes next to each background well, and click **Save**. (See Figure 3.)

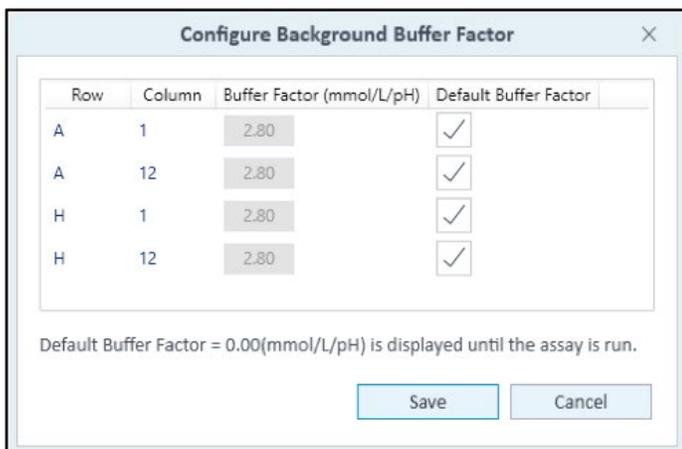


Figure 3. Configure background buffer factor

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

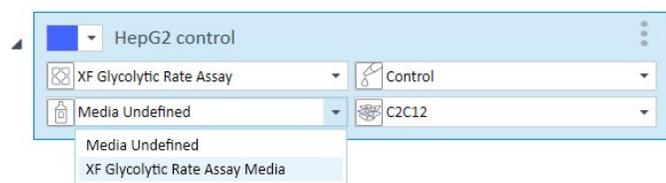


Figure 4 Media drop-down menu

- When finished, click **Apply**.
- The kinetic graph and scatter plot will display PER data when selected using the **Rate** drop-down menu.
- 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) for guidance.

- Open the assay result file in **Wave** software.
- Click **Modify**.
- Click **Add** next to **Assay Media**.
- In the Edit Assay Medium window, enter the **Buffer Factor** in the field provided.
- Next, click **Assay Media** again to configure Background Well Buffer Factor.
- Click **Configure**.
- Under **Buffer Factor (mmol/pH)**, type in the same Buffer Factor value and click **Save**. (See Figure 5.)

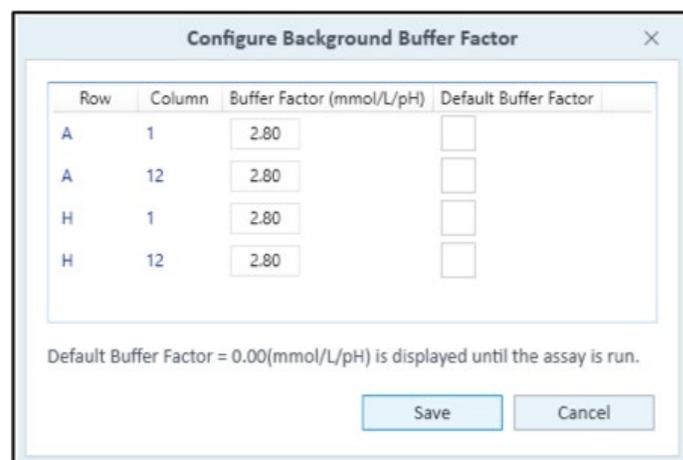


Figure 5. Type in the same buffer factor value

- Assign the **Assay Media** to each group in the group list. First click **Collapse/Expand All** to display the Group Definitions for each group.
- To select the appropriate medium for each group, use the **Media** drop-down menu.
- When finished, click **Apply**.
- The kinetic graph and scatter plot will display PER data when selected using the **Rate** drop-down menu.
- When finished with analysis, save the changes made to the result file.

Export Options for Additional Data Analysis

Calculate Glycolytic Proton Efflux Rate (glycoPER)

Wave software calculates and reports total PER only; it does not distinguish sources of extracellular acidification. If you performed the XF Glycolytic Rate Assay, you must export data to the XF Glycolytic Rate Assay Report Generator to calculate glycoPER, the rate of glycolysis-specific proton efflux. (See Figure 6.)

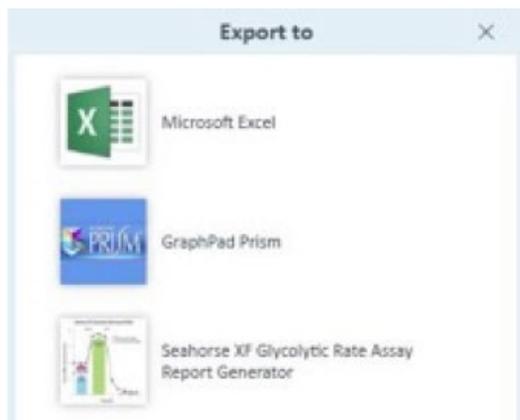
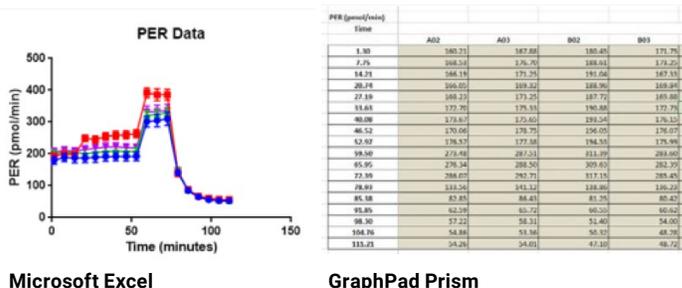


Figure 6. Export

1. Open the assay result file in **Wave** software.
2. Click **Export**.
3. Select the **XF Glycolytic Rate Assay Report Generator** from the list.
4. Modify the file name or save location if necessary, then click **Save**.

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 software and download the latest version: [www.agilent.com/en-us/products/cell-analysis-\(seahorse\)/seahorse-wave-software](http://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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