



Agilent Seahorse XF Plasma Membrane Permeabilizer (PMP) Quickstart Guide

For use with Agilent Seahorse XFe, XFp, and XF Analyzers

Product Description 2

Mitochondrial Assay Solution 4

References 6

Product Use and Limitations, Warranty, Disclaimer 7

Kit part number 102504-100

Product Description

The Seahorse XF Plasma Membrane Permeabilizer (PMP) (p/n 102504-100) is an engineered, recombinant cytolysin protein that has the ability to form pores in cellular plasma membranes via oligomerization. Unlike most detergent-based permeabilizers, it targets the cellular plasma membrane selectively, leaving the mitochondrial membrane intact. The permeabilization process with this product is consistent, reliable and accurate. Therefore there is no need to perform a concentration titration for every experiment. In general, 1.0 nM PMP is sufficient for most cell types. These unique features allow to study mitochondrial function without the need to isolate mitochondria, resulting significant improvement and simplification of your workflow and consistent results for assays.

The utility of this product has been demonstrated in assessing mitochondrial respiratory complexes, measuring substrate oxidation, and other applications. For information on these applications, please visit the technical overviews on Agilent website and/or check the reference list at the end of the document.

<http://www.agilent.com/cs/library/technicaloverviews/public/5991-7157EN.pdf>

<http://www.agilent.com/cs/library/technicaloverviews/public/XF-PMP-Limited-Tech-Brief-WEB.pdf>

<http://www.agilent.com/cs/library/technicaloverviews/public/xf%20pmp%20substrate%20oxidation.pdf>

Contents

One microfuge tube containing 25 μ L of 10 μ M PMP solution in HNG buffer (50 mM Hepes, pH 7.4, 100 mM NaCl, 10% glycerol [v/v]). Each tube contains enough material for six 24-well or 96-well microplates. The reagent box contains five slots to store 4 μ L aliquots upon arrival, or after the initial thaw.

Storage conditions

Upon arrival, store at -20 °C. The PMP stock reagent has a minimum of 6 months shelf life when properly stored. Storage of diluted PMP reagent is not recommended. Multiple freeze/thaw cycles are not recommended. Make 4 μ L aliquots into fresh tubes (user supplied) upon arrival, or after the initial thaw.

Materials required (not included)

- Agilent Seahorse XF 24/96, XFe 24/96, and XFp Analyzers
- Agilent Seahorse XF/XFe/XFp FluxPak or FluxPak mini
- Mitochondrial Assay Solution (MAS)

Reagent preparation

It is recommended that PMP be thawed on ice, mixed gently, and diluted directly into MAS (+ other supplements) immediately prior to the start of the assay.

Table 1 Reagent preparation *

Reagent	Source	3X MAS	Amount for 1.0 L of 3X MAS
Mannitol	Sigma	660 mM	120.23 g
Sucrose	Sigma	210 mM	71.88 g
KH ₂ PO ₄	Sigma	30 mM	4.08 g
MgCl ₂	Sigma	15 mM	15 mL of 1.0 M solution
HEPES	Sigma	6 mM	6 mL of 1.0 M solution
EGTA	Sigma	3 mM	12 mL of 0.25 M solution
Fatty Acid Free (FAF) BSA	Sigma	0.6% (w/v)	6.0 g

* BSA can be omitted from the MAS buffer, but greater concentration of PMP will be required to achieve a similar level of permeabilization.

Mitochondrial Assay Solution

To make 1.0 L of 3X MAS

- 1 Combine the reagents in [Table 1](#) on page 3 in 750 mL dH₂O.
- 2 Bring the volume to 950 mL with dH₂O, warm to 37 °C, and ensure all reagents are completely dissolved.
- 3 Adjust the solution to pH 7.4 with KOH.
- 4 Add dH₂O to bring the final volume to 1.0 L.
- 5 Sterile filter the solution, and store it at 4 °C.

Use of 3X MAS

Use 3X MAS buffer as a stock buffer to prepare the assay medium. Combine the 3X MAS with substrates, ADP, PMP, and dH₂O to achieve 1X MAS with the appropriate final concentrations of additives. This buffer should also be used when preparing injections for an PMP experiment.

Day prior to assay

- 1 Hydrate the XF cartridge.
- 2 Plate the cells of interest on an XF cell culture microplate.
- 3 Prepare stock reagents and solutions.

Day of assay

- 1 Prepare solutions as required.
- 2 Load the XF cartridge injection ports.
- 3 Begin cartridge calibration, after cartridge calibration is complete, wash the cells once with 1X MAS, then add the appropriate final volume of prewarmed (37 °C) assay buffer to the wells.
- 4 After the final volume addition, insert the microplate into the Seahorse XF Analyzer. (Note: Unlike standard XF assays, do not incubate at 37 °C in a non-CO₂ environment.)

Assay buffer

Agilent Technologies recommends using a nonionic mannitol plus a sucrose-based buffer (MAS, composition provided in [“To make 1.0 L of 3X MAS”](#)) for permeabilization assays.

Agilent also recommends adding XF PMP + ADP + substrates of interest to the XF assay buffer after the wash step, and not as an injection during the assay. Permeabilization just before the XF assay limits the amount of time the cell is exposed to a nonionic buffer and, thus, reduces changes in cell volume and potential cell adhesion issues.

Table 2 Suggested final concentrations for XF PMP assays

Substrates	Amount
ADP	4 mM
Succinate/Rotenone	10 mM/2 μ M
Pyruvate/Malate	10 mM/0.5 mM
Glutamate/Malate	10 mM/10 mM
Palmitoyl Carnitine/Malate	40 μ M/0.5 mM

Optimization of XF PMP assays

XF PMP Concentration

Most cell types (cell lines and primary cells) work well with 1.0 nM final XF PMP, however optimization of the concentration may be required for some cell types. Agilent Seahorse recommends titrating XF PMP between 0 and 3.0 nM (for example, 0, 0.1, 0.3, 1, 2, and 3 nM) to obtain optimal XF PMP concentrations.

Strategies for more robust adherence of cells

Agilent recommends using the method discussed above for XF PMP assays, which limits exposure of the intact cell to a nonionic media, and tends to reduce issues with cell adherence. If cell adhesion remains problematic, try:

- Seeding cells at a lower density, but allowing two days of growth
- Using a plate coating
- Slightly reducing the concentration of XF PMP

Mix, wait, measure times for XFe/XF instrument commands

Mix, wait, measure times are different than typical XF assays to reduce assay time and potential for loss of cell adherence upon permeabilization. See [Table 3](#) on page 6 for recommended mix, wait, measure times for XF PMP assays.

NOTE

There is no equilibration step in the instrument command protocol.

Table 3 Recommended mix, wait, and measure times for XF PMP assay 24-well or 96-well

Command	Time (minutes)
Mix	0.5
Wait	0.5
Measure	2.0

More information on PMP can be found at [www.agilent.com/en-us/products/cell-analysis-\(seahorse\)/seahorse-xf-consumables/kits-reagents-media/seahorse-xf-plasma-membrane-permeabilizer](http://www.agilent.com/en-us/products/cell-analysis-(seahorse)/seahorse-xf-consumables/kits-reagents-media/seahorse-xf-plasma-membrane-permeabilizer).

References

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- 2 Divakaruni, A.S.; *et al.* Analysis and interpretation of microplate-based oxygen consumption and pH data. Methods Enzymol., 2014, 546:309-354
- 3 Divakaruni, A. S.; *et al.* Measuring mitochondrial function in Permeabilized Cells using the Seahorse XF Analyzer or a Clark-Type oxygen electrode. Current Protocols in Toxicology, 2014, 60: 25.2: 25.2.1 - 25.2.16

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