XF Plasma Membrane Permeabilizer (PMP)

Part# 102504-100

Quickstart Guide

For use with XF^{e} & XF Extracellular Flux Analyzers

For Research Use Only

Seahorse Bioscience

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Product Description

The XF Plasma Membrane Permeabilizer (PMP) is an engineered, recombinant cytolysin protein that has the ability to form pores in cellular plasma membranes via oligomerization. This process allows for consistent, reliable, and accurate permeabilization using a single concentration of XF PMP, 1.0 nM for most cell types. A key benefit of the XF PMP is that it does not damage the mitochondrial membrane, a characteristic of detergent-based methods.

Contents

1 microfuge tube containing 25 µl of 10 µM XF 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 µl aliquots upon arrival or after initial thaw.

Storage Conditions

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

Materials Required (not included)

- XF^{e}24/96 or XF24/96 Analyzer
- XF/XF^{e} FluxPak or FluxPak Mini
- Mitochondrial Assay Solution [MAS] (see composition below)

Reagent Preparation

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

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
KH2PO4	Sigma	30 mM	4.08 g
MgCl2	Sigma	15 mM	15 ml of 1.0 M soln
HEPES	Sigma	6 mM	6 ml of 1.0 M soln
EGTA	Sigma	3 mM	12 ml of 0.25 M soln
Fatty Acid Free (FAF) BSA	Sigma	0.6% (w/v)	6.0 g

Note: BSA can be omitted from the MAS buffer, but greater concentrations of XF PMP will be required to achieve a similar level of permeabilization.

Mitochondrial Assay Solution

To Make 1.0 L of 3X MAS

Combine reagents in table above in 750 ml dH_2O (Thermo Scientific). Bring volume to 950 ml with dH_2O, warm to 37°C and ensure all reagents are completely dissolved. Adjust solution to pH 7.4 with KOH. Add dH_2O to bring final volume to 1.0 L. Sterile filter and store at 4°C.

Use of 3X MAS

3X MAS buffer is then used as a stock buffer to prepare the assay medium. Combine the 3X MAS with substrates, ADP, PMP and dH_2O to achieve 1X MAS with the appropriate final concentrations of additives. This buffer should also be used when preparing injections for an XF PMP experiment.

Day Prior to Assay

Hydrate the XF cartridge, plate cells of interest on XF cell culture microplate, and prepare stock reagents and solutions.

Day of Assay

Prepare solutions as required, load XF cartridge injection ports, begin cartridge calibration. After cartridge calibration is complete, wash cells once with 1X MAS, then add the appropriate final volume of pre-warmed (37°C) assay buffer to the wells. After final volume addition, insert the microplate into the XF Analyzer. (Note: unlike standard XF assays, do not incubate at 37°C in a non-CO_2 environment.)

Assay Buffer

Seahorse Bioscience recommends using a non-ionic mannitol + sucrose-based buffer (MAS, composition provided above) for permeabilization assays.

Seahorse 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 non-ionic buffer and thus reduces changes in cell volume and potential cell adhesion issues.

Suggested final concentrations of ADP and other substrates for XF PMP assays

ADP	4 mM
Succinate/Rotenone	10 mM/2 µM
Pyruvate/Malate	10 mM/0.5 mM
Glutamate/Malate	10 mM/10 mM
Palmitoyl Carnitine/Malate	40 µM/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. Seahorse Bioscience recommends titrating XF PMP between 0 and 3.0 nM (e.g. 0, 0.1, 0.3, 1, 2 and 3 nM) to obtain optimal XF PMP concentrations.

- **Strategies for more robust adherence of cells**

Seahorse Bioscience recommends using the method discussed above for XF PMP assays, which limits exposure of the intact cell to a non-ionic media and tends to reduce issues with cell adherence. If cell adhesion remains problematic, then try: seeding cells at a lower density but allowing 2 days of growth, using a plate coating, and/or slightly reducing the concentration of XF PMP.

Mix, Wait, Measure times for XF^e/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 below for recommended mix, wait, measure times for XF PMP assays.

Note: there is no 'equilibration' step in the instrument command protocol.

Command	24-well or 96-well
Mix	0.5
Wait	0.5
Measure	2.0

Download MSDS Sheets for XF PMP.

http://www.seahorsebio.com/products/consumables/kits/pmp-reagent.php

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