

# Agilent Seahorse XFp T Cell Metabolic Profiling Kit

For use with XFp PDL miniplates and T cells

## One day prior to the assay (Day 1)

1. Turn on the instrument and ensure that it is thermally equilibrated to 37 °C (minimum of 5 hours).
2. Hydrate a sensor cartridge by following the detailed instructions in the XFp kit user guide.
3. Prewarm an XFp PDL miniplate in a 37 °C non-CO<sub>2</sub> incubator overnight (>5 hours).
4. Design the experiment. Create or modify the assay template.

## Day of the assay (Day 2 – see Figure 1)

1. Complete sensor cartridge hydration.
2. Prepare 20 mL of assay medium with XF supplements (Table 1). Warm up to 37 °C.
3. Perform a cell count with the sample from the culture vessel to determine the amount of cell suspension needed.
4. Centrifuge the cells and resuspend in a small volume of the assay medium.
5. Perform the cell count again to confirm the cell number. Adjust the volume to the appropriate cell concentration (see Table 2).
6. Seed **50 µL/well** onto the XFp PDL miniplate. **Do not** seed cells in volumes higher than 50 µL/well.
7. Centrifuge the plate at 200 xg for 1 minute to allow cells to attach to the bottom of the wells.

**Table 1.** Standard assay medium.

Assay Media Component	Volume (mL)	Final Concentration
Seahorse XF RPMI Medium, pH 7.4	19.4	–
XF Glucose (1 M)	0.2	10 mM
XF Pyruvate (100 mM)	0.2	1 mM
XF Glutamine (200 mM)	0.2	2 mM

**Table 2.** Standard cell seeding densities for the different types of T cells.

Cell Type	Cell Suspension (cells/mL)	Seeding Volume (µL/well)	Final Cell Density (cells/well)
Naïve	4.0 × 10 <sup>6</sup>	50	2.0 × 10 <sup>5</sup>
Activated	2.0 × 10 <sup>6</sup>	50	1.0 × 10 <sup>5</sup>

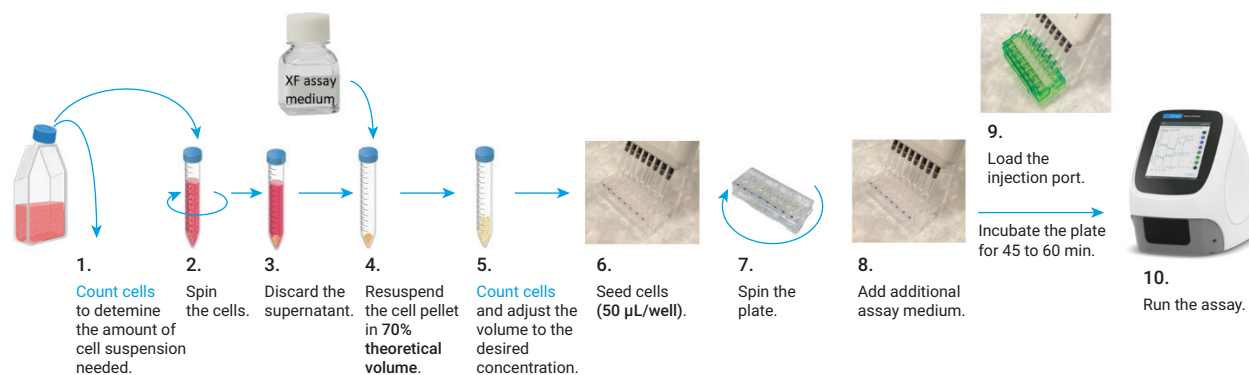
**Table 3.** Preparation of loading solutions.

Compounds	Volume to Add (mL)	Concentration (µM)
Oligomycin A	0.5	13.5
BAM15	0.5	25
Rotenone/Antimycin A	0.5	5.5

**Table 4.** Volumes of loading solutions for ports.

	Without Acute Injection		With Acute Injection	
	Loading Solution	Volume (µL)	Loading Solution	Volume (µL)
Start Well Volume		200		175
Port A	Oligomycin A	25	Test compound (8x)	25
Port B	BAM15	25	Oligomycin A	25
Port C	Rotenone/antimycin A	25	BAM15	25
Port D	–	–	Rotenone/antimycin A	25

8. Gently add additional assay medium: 150 or 125  $\mu\text{L}$ /well for assays without or with an acute injection, respectively.
9. Incubate the plate in a 37 °C non- $\text{CO}_2$  incubator for 45 to 60 minutes prior to the assay.
10. Prepare loading solutions for the compounds supplied in the kit by adding 0.5 mL of assay medium to each vial (Table 3).
11. Load 25  $\mu\text{L}$  of compound solutions to each injection port following Table 4.
12. Open the designed assay template. Click **Start Run** when you are ready.
13. Following calibration, the software will display Load Cell Plate. Click **Open Tray**, then replace the utility plate with the cell plate.
14. Ensure that the lid is removed from the cell plate, then click **Load Cell Plate** to start the assay.



**Figure 1.** Assay workflow using the Agilent Seahorse XFp T Cell Metabolic Profiling kit with an XFp PDL miniplate.

## Ordering information

Part Number	Product Description	Compatible Analyzer
103771-100	Seahorse XFp T Cell Metabolic Profiling Kit, 6 assays	- HS Mini analyzer - XFp analyzers
<b>Related Products</b>		
103576-100	Seahorse XF RPMI Medium, pH 7.4, 500 mL*	All analyzers
103577-100	Seahorse XF 1.0 M Glucose Solution, 50 mL	All analyzers
103578-100	Seahorse XF 100 mM Pyruvate Solution, 50 mL	All analyzers
103579-100	Seahorse XF 200 mM Glutamine Solution, 50 mL	All analyzers
103721-100	Seahorse XFp FluxPak (PDL Plates)	HS Mini, XFp analyzers
103722-100	Seahorse XFp PDL Cell Culture Miniplates	HS Mini, XFp analyzers
201280-100	Agilent Reservoir, 12 column, polypropylene	All analyzers
204365-100	Agilent Reservoir, 12 column, polypropylene, irradiated	All analyzers

\* This medium can also be purchased together with the supplements/substrates listed in this table as bundled products (part number 103681-100).

## Additional information

### Agilent XF Learning Center

[www.agilent.com/en/products/cell-analysis/how-to-run-an-assay](http://www.agilent.com/en/products/cell-analysis/how-to-run-an-assay)

### Technical Support

[cellanalysis.support@agilent.com](mailto:cellanalysis.support@agilent.com)

[www.agilent.com/chem](http://www.agilent.com/chem)

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