

# Agilent Seahorse XFp T Cell Metabolic Profiling Kit

For use with XFp PDL miniplates

## 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 × g 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.

Cell Type	Cell Suspension (cells/mL)	Seeding Volume (µL/well)	Final Cell Density (cells/well)
Naïve T Cell	4.0 × 10 <sup>6</sup>	50	2.0 × 10 <sup>5</sup>
Activated T Cell	2.0 × 10 <sup>6</sup>	50	1.0 × 10 <sup>5</sup>
NK Cell	5.0 × 10 <sup>6</sup>	50	2.5 × 10 <sup>5</sup>
Stimulated NK Cell	2.0 × 10 <sup>6</sup>	50	1.0 × 10 <sup>5</sup>

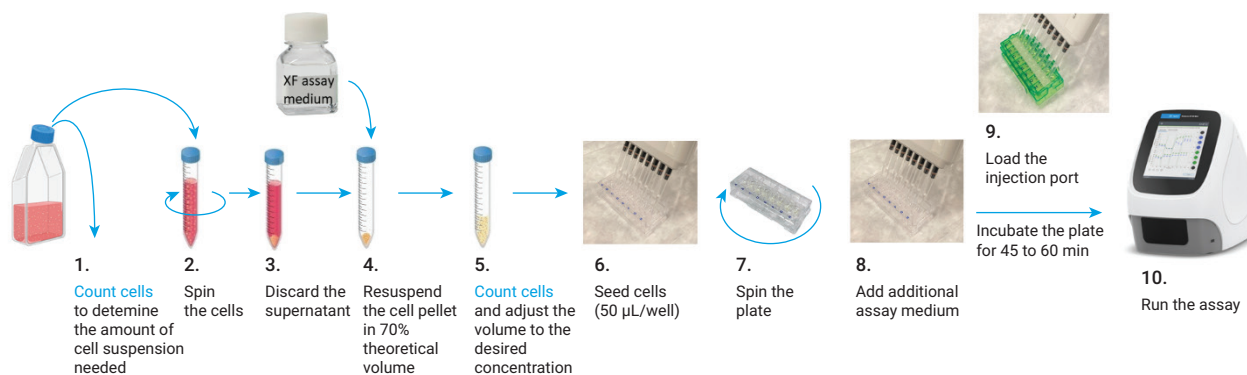
**Table 3.** Preparation of loading solutions.

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

- Gently add additional assay medium: 150 or 125  $\mu\text{L}$ /well for assays without or with an acute injection, respectively.
- Incubate the plate in a 37 °C non-CO<sub>2</sub> incubator for 45 to 60 minutes prior to the assay.
- Prepare loading solutions for the compounds supplied in the kit by adding 0.5 mL of assay medium to each vial (Table 3).
- Load 25  $\mu\text{L}$  of compound solutions to each injection port following Table 4.
- Open the designed assay template. Click **Start Run** when you are ready.
- Following calibration, the software will display Load Cell Plate. Click **Open Tray**, then replace the utility plate with the cell plate.
- 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](http://www.agilent.com)

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