

Agilent Seahorse XFp T Cell Metabolic Profiling Kit

For use with XF HS 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 XF HS PDL miniplate in a 37 °C non-CO₂ 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 **30 µL/well** onto the XF HS PDL miniplate. **Do not** seed cells in volumes higher than 30 µL/well.
7. Centrifuge the plate at 200 xg for 1 minute to allow cells to attach to the bottom of the wells.
8. Remove the silicone mask carefully following the detailed instructions in the XFp kit user guide.

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	2.3 × 10 ⁶	30	7.0 × 10 ⁴
Activated	1.0 × 10 ⁶	30	3.0 × 10 ⁴

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: 170 or 145 μL /well for assays without or with an acute injection, respectively.
- Incubate the plate in a 37 °C non-CO₂ 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 μL of compound solutions to each injection port following Table 4.
- Open designed assay template. Click **Start Run** when you are ready.
- Following calibration, the software will display Load Cell Plate. Click **Open Tray**, then replace 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.

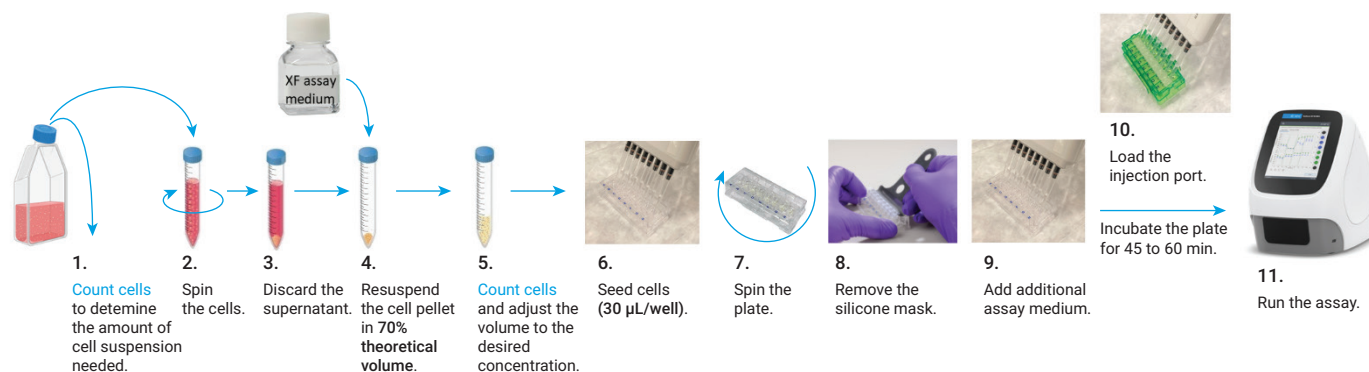


Figure 1. Assay workflow using the Agilent Seahorse XFp T Cell Metabolic Profiling kit with an XF HS PDL miniplate.

Ordering information

Part Number	Product Description	Compatible Analyzer
103771-100	Seahorse XFp T Cell Metabolic Profiling Kit, 6 assays	- HS Mini analyzer - XFp analyzers
Related Products		
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
103724-100	Seahorse XF HS Mini FluxPak (PDL Miniplates)	HS Mini analyzer
103727-100	Seahorse HS PDL Miniplates	HS Mini analyzer
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

Technical Support

cellanalysis.support@agilent.com

www.agilent.com/chem

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