

Agilent Seahorse XF T Cell Metabolic Profiling Kit

For use with XF Pro or XFe96 analyzers

One day prior to the assay (Day 1)

1. Turn on the instrument and ensure that it is thermally equilibrated to 37 °C (for a minimum of five hours).
2. Hydrate a sensor cartridge by following the detailed instructions in the XF kit user guide.
3. Prewarm a PDL cell culture microplate in a 37 °C non-CO₂ incubator overnight (>five 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 following the detailed instructions in the XF kit user guide.
2. Prepare 100 mL 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 cell count again to confirm the cell number. Adjust the volume to the appropriate cell concentration (see Table 2),
6. Seed **50 µL** per well onto XFe96 PDL plate. **Do not** seed cells in volumes higher than 50 µL.
7. Centrifuge the plate gently at 200 × g for one minute to allow cells to attach to the bottom of the wells.

Table 1. Standard assay medium for XF T Cell Persistence or Fitness assays.

Assay Media Component	Volume (mL)	Final Concentration
XF RPMI Medium, pH 7.4	97	–
XF 1.0 M Glucose Solution	1.0	10 mM
XF 100 mM Pyruvate Solution	1.0	1 mM
XF 200 mM Glutamine Solution	1.0	2 mM

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

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

Table 3. Preparation of loading solutions.

Compounds	Volume to Add (mL)	Concentration (µM)
Oligomycin A	4.0	13.5
BAM15	4.0	25
Rotenone/antimycin A	4.0	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 μL or 125 μL per well for assays without or with an acute injection, respectively.
9. Incubate the plate in a 37 $^{\circ}\text{C}$ non- 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 4 mL assay medium to each vial (see Table 3).
11. Load 25 μL of compound solutions to each injection port following Table 4.
12. Open designed assay template in Wave or XF Pro Controller. Click **Start Run** when ready. Ensure to enter SW code.
13. Following calibration, the software will display Load Cell Plate. Click **Open Tray**, then replace utility plate with the cell plate.
14. Ensure the lid is removed from the cell plate, then click **Load Cell Plate** to start the assay.

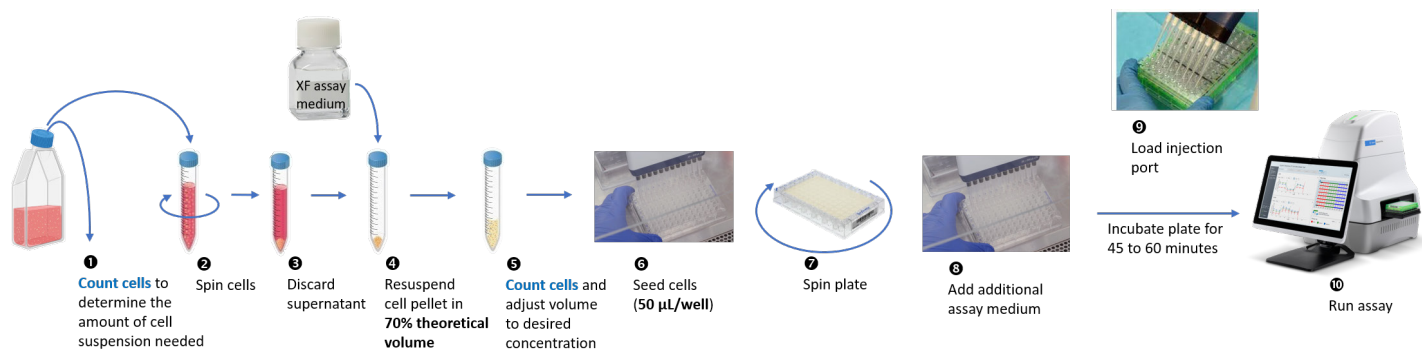


Figure 1. Assay workflow using the Agilent Seahorse XF T Cell Metabolic Profiling kit.

Ordering information

Part Number	Product Description	Compatible Analyzer
103772-100	Seahorse XF T Cell Metabolic Profiling Kit, 6 assays	XF Pro analyzer XFe96/XF96 analyzer
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
103729-100	Seahorse XFe96 FluxPak Mini (PDL Plates), 6 assays	XFe96/XF96 analyzer
103730-100	Seahorse XFe96 PDL Cell Culture Plates, 6 assays	XFe96/XF96 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).

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Additional information

Agilent XF Learning Center

www.agilent.com/en/products/cell-analysis/how-to-run-an-assay

Technical Support

cellanalysis.support@agilent.com