

Agilent Seahorse XF Hu T Cell Activation Assay Kit

One day prior to the assay (Day 1)

1. Ensure that the XF Analyzer is powered on and equilibrated to 37 °C (for a minimum of 5 hours).
2. Hydrate a sensor cartridge in sterile water in a 37 °C non-CO₂ incubator overnight.
3. Prewarm a PDL-coated cell culture microplate in a 37 °C non-CO₂ and non-humidified incubator overnight (for a minimum of 5 hours)
4. Design the experiment and create assay template in Wave

Day of assay (Day 2)

1. Complete sensor cartridge hydration by replacing water with XF calibrant (200 µL/well); incubate at 37 °C non-CO₂ incubator for 1 hour.
2. Prepare 100 mL assay medium with XF supplements (Table 1). Warm up to 37 °C.
3. Centrifuge T cells and resuspend in the assay medium to the appropriate concentration (Table 2), and seed on XFe96 or XFp PDL-coated cell culture microplate.
4. Centrifuge the plate gently (e.g. 200x g for 1 min) to allow cells to adhere to the bottom of wells.
5. Gently add 130 µL assay medium to each well.
6. Incubate the plate in a 37 °C non-CO₂ incubator for 45–60 minutes prior to the assay.
7. Prepare 10x working solution of the human CD3/CD28 activator, 2-deoxy-D-glucose (2-DG), and test compound (if desired) according to Table 3.
8. Load the 10x working solutions to injection ports following Table 4.
9. Open Wave and the pre-designed assay template. Click **Start Run** when you are ready.
10. Following calibration, Wave will display Load Cell Plate. Click **Open Tray**, then replace the Utility Plate with the Cell Plate.
11. Ensure that the lid is removed from the Cell Plate, then click **Load Cell Plate** to start the assay.

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

Table 1. Assay medium for XF Hu T Cell Activation Assay

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

Table 2. Recommended cell seeding densities for XF Hu T Cell Activation Assay

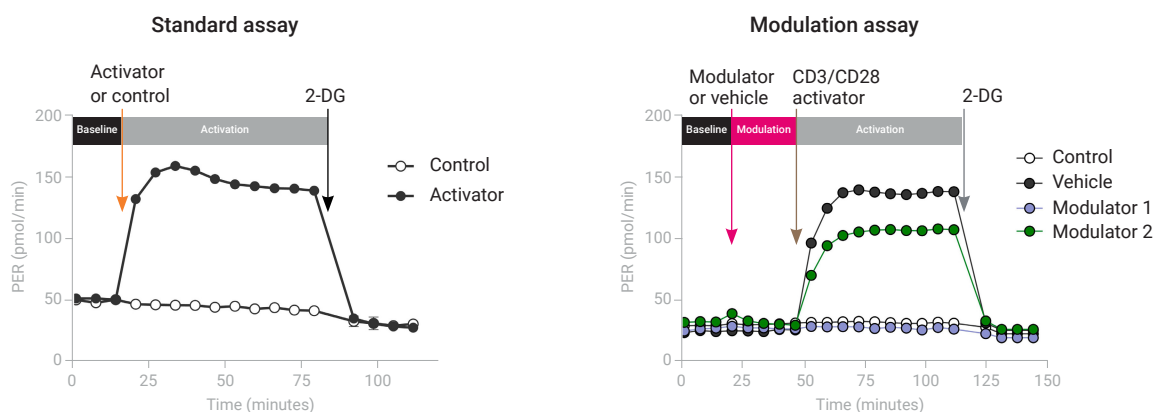
Reagent	10x Working Solution
Human CD3/CD28 Activator	1:1 mix with assay medium
2-DG	Resuspend in 3 mL assay medium
Test compound	10x concentration in assay medium

Table 3. Preparation of 10x working solutions

Port	Standard Assay		Modulation Assay	
	10x Working solution	Volume (µL)	10x Working solution	Volume (µL)
A	CD3/CD28 Activator	20	Test Compound	20
B	2-DG	22	CD3/CD28 Activator	22
C	-	-	2-DG	25

Table 4. Volumes of 10x working solutions for ports.

Agilent Seahorse XF Hu T Cell Activation Assay Profiles



Ordering information

Part Number	Product Description	Compatible Analyzer
103759-100	Seahorse XF Hu T Cell Activation Assay Kit*, 200 tests	All analyzers
103766-100	Seahorse XF Hu T Cell Activation Assay 96-Well Pack, 200 tests (contains 1x Seahorse XF Hu T Cell Activation Assay Kit, 2x XFe96 FluxPaks with PDL microplates, 1x 100 mL Calibrant)	XFe96/XF96 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
103729-100	Seahorse XFe96 FluxPak Mini (PDL Plates), 6 assays	XFe96/XF96 analyzers
103730-100	Seahorse XFe96 PDL Cell Culture Plates, 6 assays	XFe96/XF96 analyzers
103721-100	Seahorse XFp FluxPak (PDL Plates)	XF HS Mini, XFp analyzers
103722-100	Seahorse XFp PDL Cell Culture Miniplates	XF HS Mini, XFp analyzers
103724-100	Seahorse HS Mini fluxPak (PDL Plates)	XF HS Mini analyzer
103727-100	Seahorse HS PDL Miniplates	XF HS Mini analyzer

* This product does not include any FluxPaks necessary for performing T cell activation assay. Relevant XF FluxPaks can be purchased separately.

** 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 Seahorse XF Hu T Cell Activation Assay Kit User Guide:

<https://www.agilent.com/cs/library/usermanuals/public/usermanual-agilent-seahorse-xf-human-tcell-activation-assay-kit-cell-analysis-5994-1811-en-agilent.pdf>

Agilent XF Learning Center:

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

Technical Support:

cellanalysis.support@agilent.com

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