

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 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

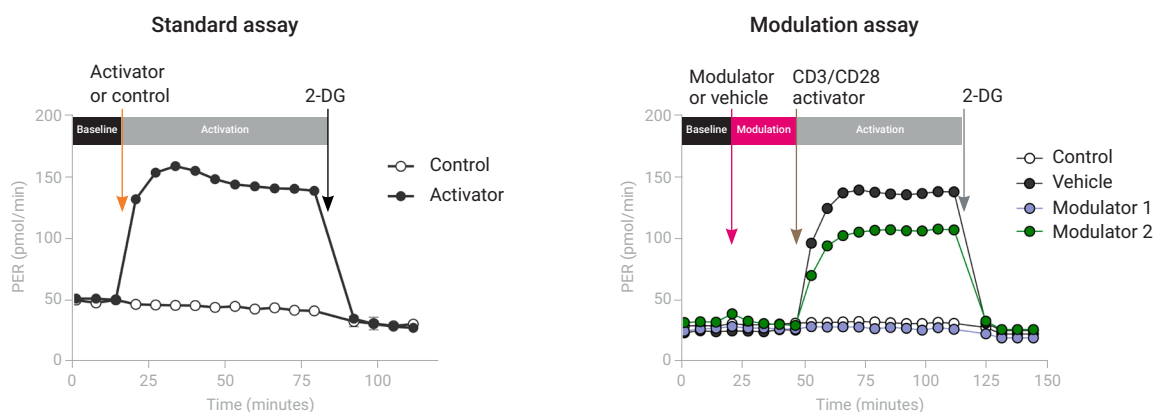
| Reagent | 10x Working Solution |
|--------------------------|-----------------------------------|
| Human CD3/CD28 Activator | 1:1 mix with assay medium |
| 2-DG | Resuspend in 3 mL assay medium |
| Test compound | 10x concentration in assay medium |

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 |

* 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:

www.agilent.com/chem/XF-Hu-T-cell-Activation-Assay-usermanual

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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DE# 274224537

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Printed in the USA, May 28, 2020
5994-1992EN