**User protocol: Drug screen with Opentrons Flex**

**Overall layout:**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
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|  | **DMSO** | **SA** | **LPZ** | **PRA** | **MAP** | **DMSO** | **MF** | **CHT** | **THF** | **SA+LPZ** | **RIF** | **DMSO** |
| **Unstim** | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| **LPS** 50ng/ml | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| **IFNa** 50ng/ml | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| **IL33** 50ng/ml | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| **GMCSF** 50ng/ml | E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| **TNFa** 50ng/ml | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| **IL246** 50ng/ml | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| **PI** | H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

**A chart of drug prescriptions

Description automatically generated with medium confidenceStart Robot setup:**

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| **A** | Lysis Buffer (260mL) | 1000µl pipette tips | Trash |
| **B** | Deepwell  block | Stims  Drugs  RPMI | 200µl  pipette tips |
| **C** | CSM  (160mL) | 1000µl pipette tips |  |
| **D** | Blood    PS | 1000µl pipette tips |  |
|  | **1** | **2** | **3** |

**A screenshot of a computer game

Description automatically generated**

**Time between blood draw and start of adding blood to the deepwell block: ~45min (<60min).**

**Preparations:**

* **A1:** Add 260mL of lysis buffer (260mL of MilliQ, 260µl of 1000X Thaw-Lyse buffer).
* **B2:** Add drug aliquots (Eppendorf’s) in the right order.

Add RPMI aliquot (15ml) to the top right.

* **C1:** Add 160mL of CSM.
* **D1:** Add 30mL of Proteomic Stabilizer to the right pipette basin.
* Start the protocol 30 min after blood draw (drug preparation steps take about 15 min).

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| Robotic: 15 min. **Drug preparation**. Final volume per well: 100µl |

**Blood draw:**

* **D1:** Add 12mL of whole blood to the left pipette basin.

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| Robotic: 5 min. **Adding blood** (95µl per well). Final volume per well: 195µl |

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| Incubator: 50 min of incubation (37°C, 5% CO2, on the vertical shaker), set timer manually. |

**Stims:**

*Stims are in clustertube strips (CT1: empty, CT7: 45µl, all other CTs 15µl)*

*Robot will dilute to 150µl total volume with RPMI, then distribute 10µl to each well.*

* **B2:** Add stim aliquots in the right order.
* **B1:** Return deepwell block from incubator.

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| Robotic: 10 min. **Stim preparation/addition**. Final volume per well: 205µl |

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| Incubator: 120 min of incubation (37°C, 5% CO2, on the vertical shaker), set timer manually. |

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| Robotic: 30 min. **Fixation** (280µl PS per well), incubation, **first round of lysis** (1000µl lysis buffer), 5 min incubation). Final volume per well: 1500µl. |

Spin at 600xg for 5 min at RT, aspirate supernatant, vortex to loosen cell pellet, and return cell block to B1.

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| Robotic: 10 min. **Second round of lysis** (1500µl lysis buffer), 5 min incubation. Final volume per well: 1500µl. |

Spin at 600xg for 5 min at RT, aspirate supernatant, vortex to loosen cell pellet, and return cell block to B1.

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| Robotic: 5 min. **CSM wash** (1500µl CSM). Final volume per well: 1500µl. |

Spin at 600xg for 5 min at RT, aspirate supernatant, vortex to loosen cell pellet.

After final aspiration: 50µl per well (stored at -80°C for barcoding). Option to continue with barcoding directly.

**Preparation of CT strips:**

**Unstim: 0µl in CT1**

150 µl PBS

**LPS (stock 1mg/ml = 1µg/µl = 1000ng/µl): 15µl in CT2 → Robot will dilute to 150µl then add 10µl per well**

Predilution: 1:100 → 10ng/µl

150ng/15µl of predilution +135µl PBS → 150ng/150µl → 10ng/10µl = 50ng/ml (in the well)

**IFNa (stock 100ng/10µl): 15µl in CT3 → Robot will dilute to 150µl then add 10µl per well**

150ng/15µl of stock+ 135µl PBS → 150ng/150µl → 10ng/10µl = 50ng/ml (in the well)

**IL-33 (stock 100ng/10µl): 15µl in CT4 → Robot will dilute to 150µl then add 10µl per well**

150ng/15µl of stock+ 135µl PBS → 150ng/150µl → 10ng/10µl = 50ng/ml (in the well)

**GMCSF (stock 100ng/10µl): 15µl in CT5 → Robot will dilute to 150µl then add 10µl per well**

150ng/15µl of stock+ 135µl PBS → 150ng/150µl → 10ng/10µl = 50ng/ml (in the well)

**TNFa (stock 100ng/10µl): 15µl in CT6 → Robot will dilute to 150µl then add 10µl per well**

150ng/15µl of stock+ 135µl PBS → 150ng/150µl → 10ng/10µl = 50ng/ml (in the well)

**IL-2/4/6 (stock 100ng/10µl):** **15µl each (45µl total) in CT7 → Robot will dilute to 150µl then add 10µl per well**

150ng/45µl combined stock +105µl PBS → 150ng/150µl → 10ng/10µl = 50ng/ml (in the well)

**PI (0.5X = 1µL/1mL): 15µl in CT8 → Robot will dilute to 150µl then add 10µl per well**

Predilution: 3µl of PI + 12µl of PBS

15µL of predilution + 135µL PBS (for 0.5X)