emulate

Protocol for Emulate Organ-Chips:

Compound Treatment Solution Preparation and Treatment

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EP152 v1.0

TITLE	DOCUMENT	VERSION
Compound Treatment Solution Preparation and Treatment	EP152	1.0
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Goals:	Key Steps:	Other Required Materials:
• Prepare compound treatment solution • Treat cells in Emulate Organ-Chips with test compounds	• Prepare compound stock solution • Prepare compound treatment solution • Treat chips with compound at test concentrations	• Compound(s) • Solvent • Analytical balance • Amber or clear glass vials • 50 mL conical tubes • Complete cell culture medium • Ice and ice bucket (for temperature sensitive compounds)

Important: Before preparing the compound for treatment of chips, carefully review the compound safety data and specification sheets and take note of the molecular weight, solubility, and stability of the compound.

Part I – Compound Stock Solution Preparation

1. Select an appropriate solvent (e.g., DMSO, water) for preparing a stock solution of the dosing compound based on recommendations from the specification sheet of that compound or its physicochemical properties. Ensure that all materials and reagents required for the preparation of the stock solution are sterile and ready. Note: Carefully select solvent as some solvents can cause solvent effects such as upregulation of CY450 drug metabolizing enzymes, or can negatively impact cell viability.

2. Whenever possible, use DMSO as solvent of choice, and maintain final concentration below 0.3% of DMSO (0.1% of DMSO preferred) to avoid any solvent effects.

3. Weigh compound into amber or clear glass vial, or use entire amount of compound in the premeasured compound vial. Note:

    - If the compound is light sensitive, we recommend preparing the stock solution in amber glass vials or in clear vials wrapped in aluminum foil.
    - If compound is toxic, or of unknown toxicity, do not weigh it in the open. Ensure that the balance is located inside a chemical fume hood and that you are using proper protective equipment for handling.

4. Calculate the volume of solvent to prepare a stock solution of the test compound based on mass / molecular weight / molarity of the compound and the desired concentration / molarity of the stock solution. Preparation of 1000X target concentration of stock solution is typically recommended as this allows you to maintain solvent at 0.1%.

5. Mix well and visually assess the stock solution to ensure the compound is completely dissolved in its solvent. Note:

    - It is best to prepare stock solutions in the biosafety cabinet with the lights turned off.
    - If the compound does not dissolve, first consider its physicochemical properties. If the compound is not temperature sensitive, consider warming it to 37°C for 10 minutes followed by a few minutes of vortexing / sonication. Alternatively, heat the solution in a 37°C water bath with sonication for 10–30 min. Caution: Ensure that all precipitates have completely re-dissolved before using the solution. Use of a fully non-dissolved compound will lead to crystallization on cells and cause non-specific toxicity and inaccurate treatment concentrations.

6. If the stock solution can be stored for future use, aliquot it into smaller volumes in glass vials. (Follow recommendations on the compound specification sheet.) Store compound stock solution according to manufacturer's recommendations, if unknown for the test compound then store at-20°C .

Part II – Compound Treatment Solution Preparation

1. Prepare as noted in the calculation table below.

2. Calculate volume of medium needed for each Organ-Chip and Pod and calculate the volumes of media, stock solution, and solvent required for each concentration of the test compound based on number of chips, flow rate, and duration of each concentration. See the calculation example below.

3. Supplement and complete medium as appropriate with required hormones and other additives.

4. Pre-warm complete media for treatment of chips at 37°C for 1 hour. Ensure that you are preparing solutions in the correct medium, as the top and bottom channels usually have different media.

5. While the medium is warming, gather 50 mL conical tubes for each dosing group and label them accordingly. (Please refer to Figure 1 below for best practices on labeling.)

6. Once the medium is warmed, collect the aliquot(s) of stock solution needed for preparation of the test compound treatment solution.

7. Following the dosing calculations, add appropriate stock solution volume to produce the treatment solution(s) in medium at the required concentrations. Note:

    - To prevent precipitation of compound from solvent while adding stock solutions to media, keep gently swirling the conical tube containing warm complete medium as you are adding stock solution to it.

8. Mix medium by vortex to ensure the stock solution is completely dissolved in the medium.

9. Keep the medium warm until you are ready to add it to the Pod™ Portable Module to begin treatment. As noted above, if the compound is light sensitive, we recommend preparing the dosing solution tube wrapped with aluminum foil. Note:

    - To minimize variability or instability, it is best to prepare all compound solutions within an hour from use.
    - You will need a vehicle control for all studies to account for any solvent effects produced by the solvent used for preparation of the stock solution of your compound.

Final Compound Concentration Required (µM)	Final DMSO Concentration Required	Stock Concentration Used to add to medium (mM)	# Chips	Total Volume of Medium for treatment of chips (mL)	Media Volume (mL)	DMSO Volume (µL)	Compound Volume (µL)
Vehicle DMSO 0.1%	0.1%	-	12	15	14.985	15	0
1	0.1%	1	12	15	14.985	0	15
10	0.1%	10	12	15	14.985	0	15
30	0.1%	30	12	15	14.985	0	15

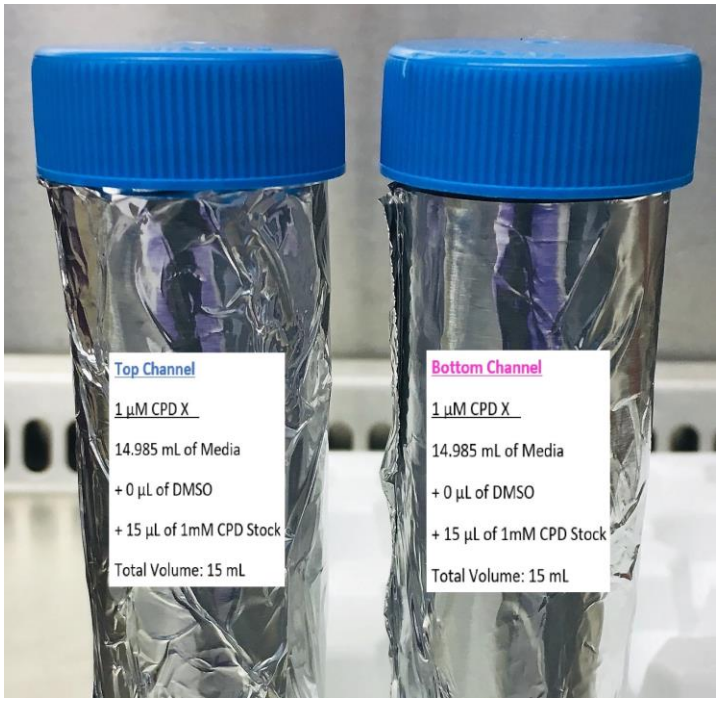

Figure 1: Best labeling practices

Part III – Treat Cells in Emulate Organ-Chips with Test Compounds

Before starting this process, ensure that all treatment solutions prepared in Part I and II of this protocol are warmed up to 37°C. Label all chips and Pods with their corresponding treatment conditions prior to adding the compound.

Note: If you have multiple collection time points in your experiment, it helps to organize your conditions so that you have 1 time point per tray. This will allow you to pause flow only on the tray from which you need to collect, while letting other trays flow uninterrupted, resulting in a more accurate assessment of elapsed time.

1. Carefully remove tray with Pods from the Zoë™ Culture Module and transfer to the biosafety cabinet. We recommend removing one tray at a time to minimize stress experienced by cells in the Organ-Chips while they are outside the incubator. Note: Ensure that the biosafety cabinet light is turned off if you are working with a light sensitive compound.

2. Fully aspirate both inlet and outlet reservoirs of each Pod, while avoiding direct contact with the Pod reservoir vias.

3. Add the calculated volume of warm, freshly-prepared treatment media to the appropriate channel, and warm, fresh media to the other channel.

4. Once all Pods have been refreshed, ensure that all trays are returned to the appropriate Zoë. Flush chips at 600 µL / hr for 5 minutes to flush dosing solution through the Pod and to prime the channels.

5. After flushing Pods and chips, carefully remove trays from Zoë one at a time. Once again, transfer to the biosafety cabinet, and aspirate accumulated media from the outlet reservoirs.

6. Return all trays to Zoë, reset the Zoë settings to the correct experimental conditions (e.g., flow rate, stretch) and note the time as the experimental start time (T=0).

7. Each outlet reservoir can be sampled independently at each timepoint following Protocol EP124 Effluent Sampling.

8. Replenish Pods with freshly prepared treatment medium at least every other day, regardless of the collection timepoints, until the end of your treatment period or experiment.