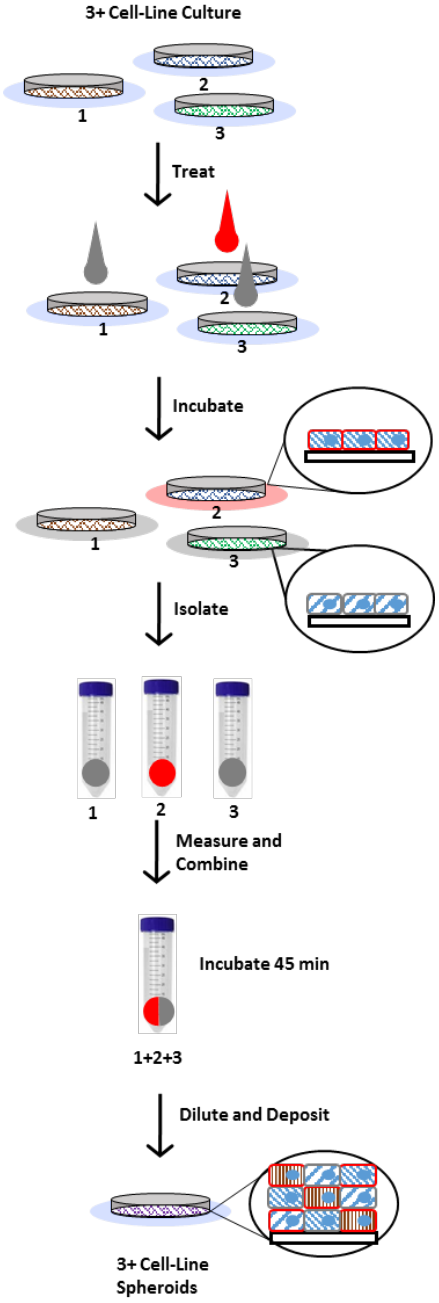
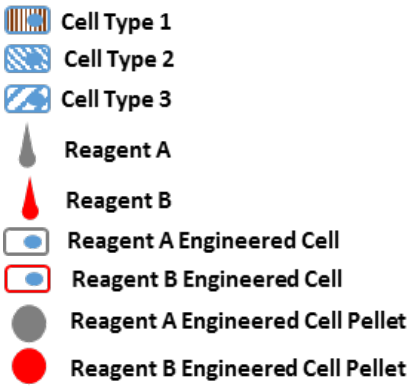
OrganoLinX





ViaGlue™ *3+ Cell-type Spheroid* Cell Assembly Protocol

Reagent A – Grey cap **Reagent B** – Red cap

1. Have three or more populations of cells grown for spheroid assembly with high density.
2. Remove the pair of reagent vials from 4°C storage and warm to room temperature.
3. Aspirate the cell growth media from the cells and wash once with PBS or fresh media or other suitable solution.
4. Aspirate the cleaning solution and replace with a minimal amount of typical growth media. Eg. 5 mL in a 10 cm culture plate, 2 mL in 6 well plate.
5. To the 5 mL of growth media (10 cm culture plate), add 250 µL of **Reagent A (5% v/v)** to cell population 1. To cell population 2 (10 cm cell plate) add 5 mL of growth media, then add 250 µL of **Reagent B (5% v/v)**. To cell population 3 add 5 mL of growth media (10 cm culture plate), then add 250uL of **Reagent A (5% v/v)**. Swirl the plates gently to mix. (To add another cell type for a total of 4 cell lines, use an aliquot of Reagent A with cell type 4).
6. Incubate the cells under optimal growth conditions for 1 hour. Eg. 37°C and 5% CO_2.
7. Aspirate the growth media containing the Reagents from the treated cell populations and wash once with PBS or fresh media or other cleaning media.
8. Remove the cell populations from the culture surface. Eg. Treat with 3 mL 0.25% trypsin for 3-5 minutes, quench with 6 mL of serum containing growth media, centrifuge and decant media.
9. With the three cell pellet populations (**1** and **2** and **3**) in separate tubes, re-suspend and measure cell density accurately (to obtain desired cell type A: cell type B ratios) to produce a desired cell composition ratio. Eg. 1:1:1.
10. In a 1.5 mL micro-tube add the calculated volumes of suspended cells together to reach a cell ratio of 1:1:1 combined.
11. Under optimal culture conditions incubate the combined suspension of **1** and **2** and **3** cells for 45 minutes to adhere through applied reagents.
12. Very gently re-suspend the pellet and dilute the formed spheroid suspension in media (recommended 100-1000X dilution).
13. Deposit the spheroids onto a desired surface, allowing the cells to incubate in optimal conditions (Eg. 37°C and 5% CO_2) undisturbed for 4-6 hours to allow cells to create natural adhesion connections.
14. Check cells under Brightfield microscopy to confirm the cells have adhered and have begun spreading out on each other.
15. Cells can be used for assays or experiments with no further manipulation.

Spheroid Assembly Considerations:

- High density depositing of cells will result in continuous tissues (further aggregation) rather than discrete spheroids.