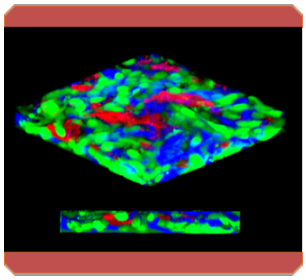
OrganoLinX



**ViaGlue™ *Thick 2 Cell Type Stacked MultiLayer Tissue* Assembly Protocol**

**Reagent A** – Grey cap

**Reagent B** – Red cap

1) Have multiple plates of two cell populations grown for cell assembly into a tissue in high densities (two 10 cm plates of cell types 1 and 2).
2) Remove the pair of vials from 4°C storage and warm to room temperature.
3) Aspirate the cell growth media 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)** and 250uL of **Reagent B (5% v/v)** to cell population 1 growth plates separately. To cell population 2 (10 cm cell plate) add 5 mL of growth media, and add 250 µL of **Reagent B (5% v/v)** and 250 µL of **Reagent A (5% v/v)**. Swirl the plates gently to mix.
6) Incubate the cells under optimal growth conditions for 1 hour. Eg. 37°C and 5% $CO_2$.
7) Aspirate the growth media from both cell population plates 2 containing the Reagents, while leaving the two treated cell population 1 plates under growth conditions.
8) Immediately remove the cell population 2 from the culture plate surfaces. 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 two cell pellet populations (**2** and **2**) in separate tubes. The cell density should be measured accurately (to obtain a desired cell A: cell B ratio) if two different cell lines are use, to produce a tissue with specific cell composition ratios.
10) Combine and mix **2** and **2**, then deposit the cells onto the 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 adhere and create natural adhesion connections.
11) Check cells under Brightfield microscopy to confirm the cells have adhered and have begun spreading out on each other.
12) Add additional amounts of media to keep tissues healthy and covered.
13) After 6 hours or overnight, remove the cell **1** and **1** populations from growth conditions and remove from the culture plate surfaces. 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.
14) With the two cell pellet populations (**1** and **1**) in separate tubes. The cell density should be measured accurately (to obtain a desired cell A: cell B ratio) if two different cell lines are use, to produce a tissue with specific cell composition ratios.
15) Combine and mix **1** and **1**, then deposit the cells onto the previously deposited cell population 2 tissue, then incubate in optimal conditions (Eg. 37°C and 5% $CO_2$) undisturbed for 4-6 hours to allow cells to adhere and create natural adhesion connections.
16) Check cells under Brightfield microscopy to confirm the cells have adhered and have begun spreading out on each other.
17) Add additional amounts of media to keep tissues healthy and covered.

18) Cells can be used for assays or experiments with no further special manipulation.

***Tissue Assembly Considerations:***

- High amounts of cells are necessary to produce 3D tissue in the prescribed area of the culture well.
- For wells with approx. 1cm$^2$ surface area, a combined 4 cell populations of approximately 18 million cells are required for 9-12 cell layers (40-70 µm thick tissue). Cells not seeded with high enough density will not result in continuous layers but rather separate spheroids on the culture surface.