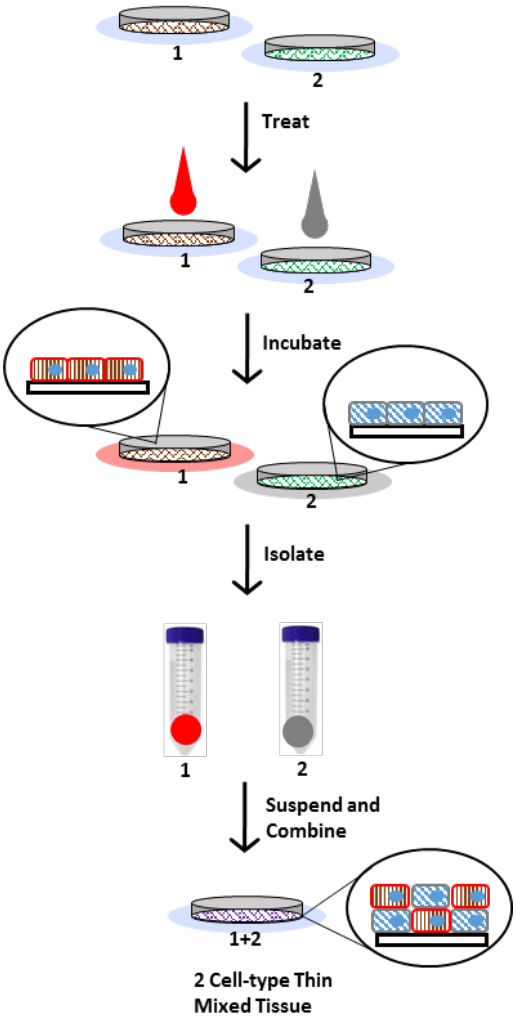
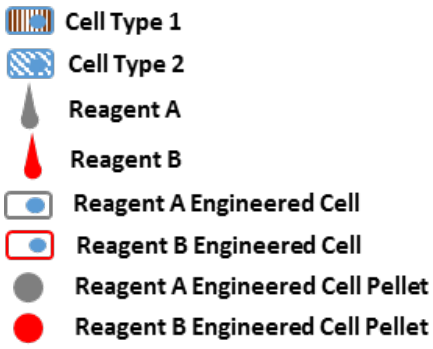
OrganoLinX

ViaGlue™ Mixed 2 cell-type Tissue Assembly Protocol

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





1. Have two populations of cells grown for cell assembly into a tissue in high densities.
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)** to cell population. To cell population 2 (10 cm cell plate) add 5 mL of growth media, then add 250 µL of **Reagent B (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 containing the Reagents from the treated cell populations and wash once with PBS, fresh media or other cleaning media.
8. Immediately remove the cells from the culture plate 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 two cell pellet populations (A and B) in separate tubes, the cell density should be measured accurately (to obtain a desired cell A: cell B ratio) to produce a tissue with specific cell composition ratios.
10. The calculated volume of cell suspensions are combined and gently agitated.
11. 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 create natural adhesion connections.
12. Check cells under Brightfield microscopy to confirm the cells have adhered and have begun spreading out on each other.
13. Add additional amounts of media to keep tissues healthy and covered.
14. 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. 1 cm^2 surface area, a combined 2 cell populations of approximately 9 million cells are required for 4-5 cell layers (25-35 µm thick tissue).
- Cells not seeded with high enough density will not result in continuous layers but rather separate spheroids on the culture surface.