

## Mixing Cells Protocol

# Chitosan Series

*This is a suggested procedure, please adjust according to your experimental needs.*

### Protocol aim

The aim of this protocol is to provide instructions for mixing cells with any bioink within the Chitosan Series, both small volumes below 2 mL and large volumes up to 10 mL.

### Material needed

- Cells in suspension
- Culture medium
- Chitosan bioink variation\*
- Pipette and pipette tips
- 3 mL syringes with Luer lock connections
- Female/female Luer lock adaptor\*
- OR
- CELLMIXER\*

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\* The product can be purchased in the CELLINK shop at [www.cellink.com/shop/](http://www.cellink.com/shop/).

### Protocol

This protocol is adjusted for mixing either 1 mL or 3 mL of bioink with cells to a final cell concentration of 10 million cells/mL bioink. The same protocol can be followed for other quantities and cell concentrations with appropriate recalculations

Step	Title	Material	Description
1	Prepare cell suspension	<ul style="list-style-type: none"><li>- Cells</li><li>- Culture medium</li></ul>	<p><b>If preparing for quantities &lt;2 mL of Chitosan bioink.</b></p> <ul style="list-style-type: none"><li>- Resuspend 11 million cells in 100 <math>\mu</math>L cell culture medium if mixing with 1 mL bioink.</li><li>- Move on to Step 2a.</li></ul> <p><b>If preparing for quantities &gt;2 ml of Chitosan bioink.</b></p> <ul style="list-style-type: none"><li>- Resuspend 33 million cells in 300 <math>\mu</math>L cell culture medium if mixing with 3 mL bioink.</li><li>- Move on to Step 2b.</li></ul>

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2a	- Mixing small volumes of Chitosan bioink with cells	- 1 mL Chitosan bioink - Cells in suspension - 3 mL syringes - Female/female Luer adapters	<p>Mix ten parts of bioink with one part of cell suspension without introducing air bubbles to the mixture.</p> <ul style="list-style-type: none"> <li>- Transfer the 100 <math>\mu</math>L cell suspension to a 3 mL syringe using a Female/female Luer lock adaptor.</li> <li>- Transfer 1 mL of bioink to a 3 mL syringe using a Female/female Luer lock adaptor.</li> <li>- Immediately refrigerate the remaining Chitosan bioink.</li> <li>- Attach the bioink syringe to the syringe with cell suspension.</li> <li>- Carefully mix the bioink with the cell suspension by gently pushing the bioink back and forth between the syringes.</li> <li>- Transfer the cell-containing bioink back to the cartridge and cap it.</li> <li>- <i>Video link for a detailed illustration on how to perform the mixing process:</i> <a href="https://www.youtube.com/watch?v=NmdOTNLRV-Q">https://www.youtube.com/watch?v=NmdOTNLRV-Q</a></li> </ul> <p>Note: To avoid an air gap when mixing the bioink and the cell suspension, carefully pre-fill the luer lock adaptor with Chitosan bioink before attaching the syringe with the cell suspension.</p>
2b	Mixing larger volumes of Chitosan bioink with cells	- 3 mL Chitosan bioink - Cells in suspension - CELLLMIXER	<p>Mix ten parts of bioink with one part of cell suspension without introducing air bubbles to the mixture.</p> <ul style="list-style-type: none"> <li>- Transfer the 300 <math>\mu</math>L of cell suspension to the 1 mL cell syringe (PART 1) using a Female/female Luer lock adaptor.</li> <li>- Transfer 3 mL of Chitosan bioink to the 12 mL syringe (PART 2 ) using a Female/female Luer lock adaptor.</li> <li>- Clip both syringes to the Dispensing unit (PART 3).</li> <li>- Connect the two syringes to the Mixing unit (PART 4), then connect the Empty cartridge (PART 5) to the Mixing units other side.</li> <li>- Apply gentle pressure onto the Dispensing unit to mix the content of both syringes and transfer it into the empty cartridge.</li> <li>- <i>Video link for a detailed illustration on how to perform the mixing process using the CELLLMIXER:</i> <a href="https://www.youtube.com/watch?v=CmSYL1-oltI">https://www.youtube.com/watch?v=CmSYL1-oltI</a></li> </ul>

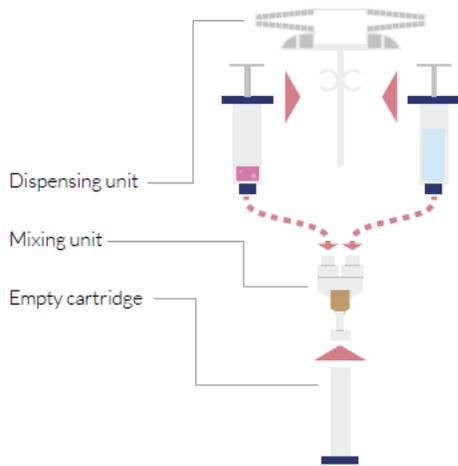


Figure 1. Illustration of how to assemble the CELLMIXER components.

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