

## Mixing Cells Protocol

# GelX 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 how to mix cells with any ink within the GelX bioink series, both small volumes below 2 ml and large volumes up to 10 ml. The GelX Series includes GelXA, GelXA BONE, GelXA FIBRIN, GelXA LAMININK, GelXA SKIN and GelXG.

### Material needed

- Cells in suspension
- Cell culture medium
- GelX 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/store/](http://www.cellink.com/store/).

**KEEP THE INK PROTECTED FROM LIGHT IF TRANSFERRED FROM THE ORANGE UV PROTECTED CARTRIDGES TO AVOID CROSSLINKING BEFORE PRINTING. THE PHOTOINITIATOR IS SENSITIVE TO REPEATED OR PROLONGED EXPOSURE TO HEAT.**

### 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. For other quantities and cell concentrations recalculations need to be made but the same protocol can be followed.

Step	Title	Material	Description
1	Preheat bioink	- GelX bioink variation	- Heat the bioink in the amber cartridge to 33-37°C until it is liquid. The heating can be performed in a pneumatic printhead, water bath or incubator.

2	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 GelX bioink.</b></p> <ul style="list-style-type: none"> <li>- Resuspend 11 million cells in 100 µl cell culture medium if mixing with 1 ml bioink.</li> <li>- Move on to Step 3a.</li> </ul> <p><b>If preparing for quantities &gt; 2 ml of GelX bioink.</b></p> <ul style="list-style-type: none"> <li>- Resuspend 33 million cells in 300 µl cell culture medium if mixing with 3 ml bioink.</li> <li>- Move on to Step 3b.</li> </ul>
3a	Mixing small volumes GelX bioink with cells	<ul style="list-style-type: none"> <li>- 1 ml GelX bioink variation</li> <li>- Cell suspension</li> <li>- 3 ml syringes</li> <li>- Female/female luer adapters</li> </ul>	<p>At this point, mix ten parts bioink with one part cell suspension, taking care not to introduce air bubbles to the mixture.</p> <ul style="list-style-type: none"> <li>- Transfer the 100 µl cell suspension to a 3 ml syringe wrapped in aluminium foil, using a female/female luer lock adaptor.</li> <li>- Transfer 1 ml of bioink to a 3 ml syringe wrapped in aluminium foil, using a female/female luer lock adaptor.</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.</li> <li>- Transfer the cell containing bioink back to the amber 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 GelX bioink before attaching the syringe with the cell suspension.</p>
3b	Mixing larger volumes GelX bioink with cells	<ul style="list-style-type: none"> <li>- 3 ml GelX bioink variation</li> <li>- Cell suspension</li> <li>- CELLLMIXER</li> </ul>	<p>At this point, mix ten parts bioink with one part cell suspension, taking care not to introduce air bubbles to the mixture.</p> <ul style="list-style-type: none"> <li>- Transfer the 300 µl cell suspension to a 1 ml cell syringe (PART 1) using a female/female luer lock adaptor.</li> <li>- Transfer 3 ml of 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> </ul>

			<ul style="list-style-type: none"> <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 into the empty cartridge.</li> <li>- <i>Video link for a detailed illustration on how to perform the mixing process using the CELLMIXER:</i>  <a href="https://www.youtube.com/watch?v=CmSYL1-oltI">https://www.youtube.com/watch?v=CmSYL1-oltI</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 GelX bioink before attaching the syringe with the cell suspension.</p>
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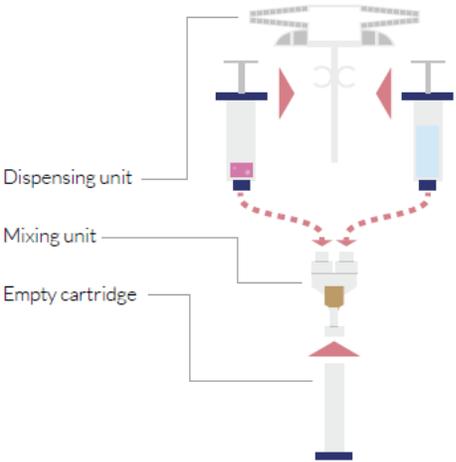


Figure 1. Illustration of how to assemble the CELLMIXER components.