

Neutralization and Casting Protocol

Collagen PREMIUM

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

Protocol aim

The aim of this protocol is to provide instructions for neutralization and casting of the Collagen PREMIUM using the INKREDIBLE, INKREDIBLE+, or BIO X, with and without cells. It covers both dispensing of the biomaterial with encapsulated cells and post seeding of cells on casted gels. Collagen PREMIUM is crosslinked through thermal induced gelation. This protocol was optimized for undiluted Collagen PREMIUM as well as for a 10+1 cell suspension diluted version. Changing the concentration of solution to cell suspension ratio will change the gelation time.

Material needed

- Collagen PREMIUM*
- Collagen PREMIUM Neutralization Solution*
- Cartridges, 3cc*
- BIO X* or INKREDIBLE series* 3D Bioprinter
- Sterile conical bioprinting nozzles*

- Cells + culture medium
- 3 mL syringes with Luer lock connections
- Female/female Luer lock adaptor*
- CELLMIXER*

*The product can be purchased in the CELLINK store at www.cellink.com/store/.

Protocol

This protocol works best with the BIO X and the Temperature-controlled printhead. If using the INKREDIBLE+ system, the dispensing procedure should be performed fast to prevent the solution from warming and gelling in the cartridge prior to dispensing.

Protocol for casting of Collagen PREMIUM with embedded cells

Step	Title	Material	Description
1	Cool material	<ul style="list-style-type: none"> - Bottle of Collagen PREMIUM - Bottle of Neutralization Solution - Ice bath 	Place the bottle of Collagen PREMIUM and the bottle of Neutralization Solution on ice to keep cool.
2	Neutralization	<ul style="list-style-type: none"> - 3 mL syringes, two units - Female/female Luer lock adaptor 	<p>Transfer equal volumes of Collagen PREMIUM and Neutralization Solution into two different 3 mL syringes.</p> <p>Note: Remove the syringe plunger, cap the syringe and pour the solution in the syringe. Insert the plunger, flip the syringe and release the tip cap to evacuate the air.</p> <ul style="list-style-type: none"> - Connect the syringes via a female/female Luer lock adaptor and mix thoroughly back and forth. - Work fast to prevent materials from warming.
3	Mix Collagen PREMIUM with cells	<ul style="list-style-type: none"> - Cell suspension in syringe - Cooled Collagen PREMIUM solution - Female/female Luer lock adaptor 	<p>If not casting with cells, move directly to step 4.</p> <p>Mix ten parts of cooled Collagen PREMIUM solution with one part of cell suspension without introducing air bubbles to the mixture. For detailed instructions see the <i>Mixing cells Protocol Collagen Series</i>.</p> <ul style="list-style-type: none"> - Attach the Collagen PREMIUM solution syringe to the syringe with cell suspension, with a female/female Luer lock adaptor. - Carefully mix the solutions with the cell suspension by gently pushing the solutions back and forth between the syringes. <p>Note: Suggested cell suspension density is 1×10^6 cells/mL to 5×10^6 cells/mL of Collagen PREMIUM.</p> <p>Note: To avoid an air gap when mixing the solution and the cell suspension, carefully pre-fill the Luer</p>

			lock adaptor with Collagen PREMIUM solution before attaching the syringe with the cell suspension. - If preparing for quantities > 2ml of Collagen PREMIUM, it is recommended to use the CELLMIXER.
4	Load the cartridge	- Cartridge, 3cc	- Transfer the solution (with or without cells) to the cartridge and cap it. - If using the BIO X, pre-cool the Temperature controlled printhead to 15°C and pre-heat the print bed to 37°C. If using the INKREDIBLE-series, cool down the cartridge for 5 min if needed.
5	Cool the cartridge	- Cartridges, 3cc loaded with Collagen PREMIUM - Sterile conical bioprinting nozzles	- Place the cartridge in the printhead and cap with a bioprinting nozzle of choice.
6	Casting	- Bioprinter (BIO X or INKREDIBLE series)	- Dispense the required volume of solution in a mould or well plate. Note: If waiting too long between extrusions, the solution can warm in the nozzle causing it to clog. If this occurs, replace with new nozzle.
7	Thermal crosslinking		Collagen PREMIUM thermally crosslinks. - Incubate the constructs at 37°C until gelation occurs, approximately 10-15 min for volumes below 100 µl. For larger constructs, prolong the incubation to 30 min. The BIO X print bed heated to 37°C can be alternatively used. Note: To verify the crosslinking is sufficient, add 37°C media to one construct and observe that it doesn't dissolve.
8	Incubation	- Cell culture medium	- Add the desired medium to submerge the constructs. - Incubate the constructs in cell culture medium in standard culture conditions (37°C, 5% CO ₂ and 95% relative humidity) or according to your application.

Protocol for cell seeding on casted Collagen PREMIUM

Step	Title	Material	Description
1	Cool material	<ul style="list-style-type: none"> - Bottle of Collagen PREMIUM - Bottle of Neutralization Solution - Ice bath 	<ul style="list-style-type: none"> - Place the bottle of Collagen PREMIUM and the bottle of Neutralization Solution on ice to keep cool.
2	Neutralization	<ul style="list-style-type: none"> - 3 mL syringes, two units - Female/female Luer lock adaptor 	<ul style="list-style-type: none"> - Transfer equal volumes of Collagen PREMIUM and Neutralization Solution into two different 3 mL syringes. - Note: Remove the syringe plunger, cap the syringe and pour the solution in the syringe. Insert the plunger, flip the syringe and release the tip cap to evacuate the air. - Connect the syringes via a female/female Luer lock adaptor and mix thoroughly back and forth. - Work fast to prevent materials from warming.
3	Load the cartridge	<ul style="list-style-type: none"> - Cartridge, 3cc 	<ul style="list-style-type: none"> - Transfer the Collagen PREMIUM solution to the cartridge and cap it. - If using the BIO X, pre-cool the Temperature controlled printhead to 15°C and pre-heat the print bed to 37°C. If using the INKREDIBLE-series, cool down the cartridge for 5 min if needed.
4	Cool the cartridge	<ul style="list-style-type: none"> - Cartridges, 3cc loaded with Collagen PREMIUM - Sterile conical bioprinting nozzles 	<ul style="list-style-type: none"> - Place the cartridge in the printhead and cap with a bioprinting nozzle of choice.
5	Casting	<ul style="list-style-type: none"> - Bioprinter (BIO X or INKREDIBLE) 	<ul style="list-style-type: none"> - Dispense the required volume of solution in a mould or well plate. - Note: If waiting too long between extrusions, the solution can warm in the nozzle causing it to clog. If this occurs, replace with new nozzle.
6	Thermal crosslinking		<p>Collagen PREMIUM thermally crosslinks.</p> <ul style="list-style-type: none"> - Warm the construct to 37°C until gelation occurs, approximately 10-15 min for volumes below 100 µl. For larger constructs, prolong the incubation to 30 min. The BIO X print bed heated to 37°C can be alternatively used.

7	Cell seeding	- Cell suspension	- Dispense the cell suspension in the middle of the casted hydrogel. Suggested cell suspension density: 2×10^4 cells/cm ² to 5×10^4 cells/cm ² - A highly concentrated cell suspension is suggested.
8	Incubation	- Cell culture medium	- Incubate for 1-2 h. - Add the desired medium to submerge the constructs and place in incubator. - Incubate the constructs in cell culture medium in standard culture conditions (37°C, 5% CO ₂ and 95% relative humidity) or according to your application.