Isolating Cells from Construct Protocol

CELLINK Bioink

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 isolate cells from a 3D bioprinted and crosslinked construct of CELLINK® Bioink. The cells can subsequently be used for flow cytometry.

Material needed

- Cell-laden 3D bioprinted construct
- PBS with 2 mM EDTA and 0.5% BSA
- 1 ml pipette and pipette tips
- Cell strainer (40 µm nylon)
- 50 mL Falcon tube
- Centrifuge
- Buffer for flow cytometry

Protocol

This protocol is for constructs printed in 96-well plate, for other sizes change the recommended volumes accordingly. Constructs of the same condition can be pooled into the same suspension. This protocol is a courtesy of Lisa Oliver, PhD, at the University of Nantes, France.

Step	Title	Material	Description
1	Dissociation	- PBS, EDTA and BSA - Cell laden constructs - 1 ml pipette and pipette tips	- Prepare the PBS with 2 mM EDTA and 0.5% BSA. - Remove cell culture medium from the construct. - Add 100 µl of buffer to each well. - Gently dissociate the cell-laden construct by pipetting 5-10 times with a 1 ml pipette. Note: Take care not to produce air bubbles .
2	Cell isolation	- Cell strainer, 40 µm nylon - 50 mL Falcon tube	- Place the cell strainer over the centrifuge tube. - Transfer the cell suspension to the cell strainer. - Wash the cell strainer 3 x with 2 ml buffer. - Remove the cell strainer and cap the Falcon tube.

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		- Centrifuge	- Centrifuge the recovered cell suspension at 2000 RPM for 5 minutes.
3	Flow cytometry preparation	- Buffer for flow cytometry	- Remove the supernatant. - Resuspend the cell pellet in a buffer compatible for flow cytometry and run the flow cytometry analysis according to your protocol of choice.

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