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Cell Recovery Protocol

Cell Collect A

This is a suggested procedure, please adjust according to your experimental needs. To maintain the sterility of the product, work under sterile conditions.

Protocol aim

This protocol provides instructions for the use of Cell Collect A, an enzyme-based proprietary formulation, to enhance cell isolation or reduce viscosity of alginate-based bioinks. The proposed method allows for digesting of bioinks either fully or partially and harvesting cells that can subsequently be used for cell viability analyses, RNA isolation, protein extraction, western blots, and qPCR among other downstream applications. Under sterile conditions, recovered cells can be re-plated for culture or used for analysis.

Materials needed

- Cell Collect A*
- Sterile PBS (1X)
- Cell-laden bioprinted constructs
- Cell shaker
- Sterile 1 M NaOH *(optional)*
- Cell culture media (*optional*)
- Cell strainer (40-70 µm nylon)
- Sterile 1-5 mL centrifuge tubes
- Centrifuge

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

Protocol

Step	Title	Material	Description
1	Diluting Cell Collect A (*optional*)	- Cell Collect A - Sterile PBS (1X)	- Dilute Cell Collect A in PBS to achieve desired concentration. Note: See Table 1 for recommended concentrations.
2	Dissociation	- Cell-laden bioprinted constructs	- Remove cell culture medium from construct(s). - Add a 10:1 volume ratio of Cell Collect A to bioink to desired well(s).

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		- Cell shaker - NaOH *(optional)* - Sterile PBS (1X) (*optional*) - Cell culture media (*optional*)	- Place the entire well plate on a cell shaker at 4°C for ~30 min, or until fully dissolved. Note: Gentle disintegration with a large orifice pipette tip is required to fully break down constructs. - For partial degradation, Cell Collect A can be inhibited by adding a 1:10 volume ratio of 1 M NaOH to Cell Collect A for 15 min, washing once and carefully replacing it with fresh media. Note: To slow cell signaling pathways, it's recommended to use Cell Collect A at 4°C. If desired, 3D constructs in Cell Collect A can be incubated at room temperature or 37°C.
3	Cell Isolation	- Cell strainer - Centrifuge tube(s) - Sterile PBS (1X)	- Place a cell strainer over a centrifuge tube and wet the bottom of the strainer with sterile PBS to facilitate flow-through. - In the same tube, use the cell strainer to filter the dissolved ECM-cell suspension.
4	Centrifuge	- Centrifuge	- Centrifuge the collected cell suspension at 400 g for 3-4 min. - Remove supernatant. - Cell pellet is ready to use for desired applications.

Table 1. Recommended Cell Collect A concentrations for partial or complete digestion of bioink droplets

Digestion	Cell Collect A dilution	Bioink droplet volume (µL)	Minimum time to digest at 4°C (min)
Partial**	Diluted more than twice	10-100	15–30
Complete	Non-diluted	10-25	15–30
		25-100***	30–60

***Partial bioink digestion may be desired for improved proliferation, porosity or delivery of small molecules. For this, diluted concentrations of Cell Collect A can be added to constructs.*

****For bioprinted constructs larger than 100 µL, higher concentrations of Cell Collect A may be required.*