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Reconstitution and Cell Recovery Protocol

Cell Collect G

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

Protocol aim

This protocol provides instructions for the preparation and use of Cell Collect G, an enzymatic lysis reagent for cell isolation or degradation of collagen- and gelatin-based bioinks. The proposed isolation method allows for a direct analysis of cell viability after bioprinting as well as other downstream applications. Under sterile conditions, recovered cells can be re-plated for culture or used for analysis.

Material needed

- Cell Collect G*
- Light-protected Falcon tube (provided)
- Syringe filter (provided)
- 50 mL HBSS (1X) or other salt balanced solution
- Cell-laden bioprinted constructs
- Centrifuge
- Cell shaker
- Sterile 2 mM EDTA or cell culture medium
- Sterile 1-5 mL centrifuge tubes
- Cell strainer (40-70 µm nylon)

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

Reconstitution and Bioink Digestion protocol

Step	Title	Material	Description
1	Reconstitution	- Cell Collect G - HBSS (1X) or other salt balanced buffer - Light-protected Falcon tube - Syringe Filter - Centrifuge tubes (*optional*)	- Add 50 mL of HBSS to Cell Collect G while protecting from light. Shake well, or until the powder is fully dissolved to the original concentration. - Use the provided syringe filter to sterilize the combined solution, and transfer into a light-protected Falcon tube. Note: Immediately store excess solution as aliquots in centrifuge tubes at -20°C. Thaw aliquot of Cell Collect G solution for 2-3 h in fridge (for ≈10 mL).

2	Digestion	- Cell-laden bioprinted constructs - Cell shaker	- Remove cell culture medium from construct(s). - Add a 10:1 volume ratio of Cell Collect G to bioink to desired well(s). - 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. Note: To slow cell signaling pathways, it's recommended to use Cell Collect G at 4°C. If desired, 3D constructs in Cell Collect G can be incubated at room temperature or 37°C.
3	Cell Isolation	- Cell strainer - Centrifuge tube(s) - 2 mM EDTA or cell culture media	- Place a cell strainer over a centrifuge tube and wash it once with cell culture media or 2 mM EDTA. - 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 rcf for 3-4 min. - Remove supernatant. - Cell pellet is ready to use for desired applications.

Table 1. Recommended Cell Collect G concentration for bioink digestion

Bioink volume	Cell Collect G dilution	Minimum time to digest at 4°C (min)
10-25 µL	Diluted twice	30–45
100 µL	Original	60–120
400 µL	Original	120–180