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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 and/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. If sterilizing the Cell Collect G and used under sterile conditions, recovered cells can be re-plated for culture or used for analysis.

Materials needed

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

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

Protocol

1. Reconstitution

MATERIAL

Cell Collect G powder

HBSS (1X) or other salt balanced buffer

Light-protected Falcon tube

Syringe filter

DESCRIPTION

- Add 50 mL of HBSS to the Cell Collect G powder 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 in fridge.

2. Digestion

MATERIAL

Cell-laden bioprinted constructs

Cell Collect G solution

Cell shaker

DESCRIPTION

- Remove cell culture medium from construct(s).
- Add enough Cell Collect G to cover the construct(s).
- Place the entire well-plate on a cell shaker at 4°C for ~30 min or until fully dissolved, see Table 1 for recommended incubation times and possible dilutions.

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.

Table 1. Recommended Cell Collect G incubation times 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

3. Cell isolation

MATERIAL

Cell strainer

Centrifuge tube(s)

Cell culture media or 2 mM EDTA

DESCRIPTION

- 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.
- Centrifuge the collected cell suspension at 200 *g* for 3-4 min.
- Remove supernatant.
- Cell pellet is ready to use for desired applications.