RASTRUM

Protocol

Cell Retrieval from RASTRUM™ Matrices

Document Number | WET.BCM.0009.PRO.v4.03
Date of issue | May 2023

INVENTIA
INSPIRING SCIENCE

Introduction

The recovery of encapsulated cell models is essential for the generation of valuable biological data from various downstream assays. To enable this feature, we have developed RASTRUM Cell Recovery Solution (CRS), an enzymatic reagent for the rapid retrieval of cells encapsulated in RASTRUM matrices. Recovered cellular material can be used in downstream applications for single cells (e.g. protein quantification, qPCR, flow cytometry and single-cell RNA-seq) and intact spheroids/aggregates (e.g. immunohistochemistry, seahorse, microfluidics etc.). For downstream application, such as RT-qPCR and RNA Sequencing, we recommend using our direct RNA extraction protocol.

Storage and handling of reagents

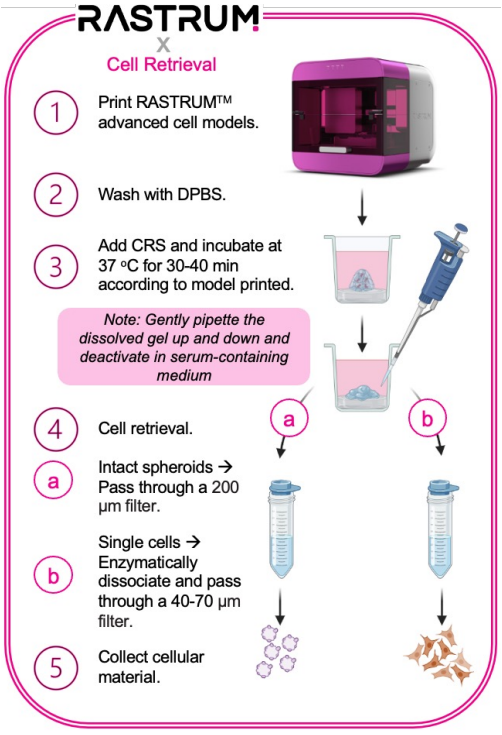
Store reagents at -20°C upon receipt. For best results, do not leave reagents at room temperature (RT) for longer than 1 hour before using it.

Note: While CRS may be freeze-thawed up to three times without loss in activity, we advise against repeated freeze-thawing of CRS.

Equipment and reagents required, but not provided

- RASTRUM advanced cell models
- RASTRUM Cell Recovery Solution
- 1 x Dulbecco's Phosphate-Buffered Saline (DPBS) solution (ThermoFisher, 14190144)
- Serum-containing media
- Cell dissociation reagent e.g. TrypLETM Express Enzyme (ThermoFisher, 12604013) or similar.
- 40 and 200 µm cell strainers (pluriStrainer Mini, 43-10040-60 and 43-10200-60) or similar
- 1.5 mL microcentrifuge tubes
- 15 mL conical tubes
- *Optional: 0.1% bovine serum albumin (BSA) dissolved in DPBS*

Graphical Protocol



Protocol

Note: CRS will dissociate cell-laden biofunctional RASTRUM matrices; however, the inert base will remain intact.
Note: This protocol should be performed inside a biosafety cabinet.

1. Thaw RASTRUM CRS for 30 min at room temperature (RT).
   Note: Batch-to-batch variation in the colour of CRS is normal.
2. Aspirate and discard media from RASTRUM advanced cell models.
   Note: For all fluid removal steps, tilting the plate at a 45-degree angle to collect liquid at the bottom edge of the well will help maximise fluid removal from the plate. When aspirating media, be careful not to pierce or scratch the encapsulated cells and inert base with your pipette tip.
3. Add 1x DPBS and incubate for 5 min at 37°C:
   - For 96-well plates, add 150 µL.
   - For 384-well plates, add 40 µL.
4. Aspirate and discard DPBS.
5. Add CRS to wells according to **Table 1** and incubate at 37°C:

Table 1. Recommended cell retrieval conditions.

Model	Matrix stiffness (kPa)	CRS (F-code)	CRS volume (µL)	Incubation time (min)
Imaging	0.7, 1.1 & 3	F234	75	30
Imaging	4.8	F235	75	40
Large Plug	0.7 & 1.1	F235	75	30
Large Plug	3 & 4.8	F235	150	40
High-Throughout	0.7 & 1.1	F235	40	30

6. Using a P1000 pipette, add 100 µL of 1x DPBS to each well and **gently** pipette up and down several times to resuspend dissociated matrix/cell mixture.
   *Note: Since this protocol can be used with both spheroid- and network-forming cell types, ensure to pipette up and down **gently** such that large matrix fragments are dissociated into small fragments and cell spheroids remain intact. Network-forming cells will dissociate into small aggregates.*
   Note: To avoid introducing bubbles into the wells during pipetting, we recommend adjusting the volume of your pipette to ~50% of the total liquid volume in the well prior to mixing the dissociated matrix/cell mixture (e.g. for Imaging Model, 90 µL; for Large Plug, 90 or 125 µL depending on CRS volume).
7. Transfer the solution into a microcentrifuge tube containing 400 µL of serum-containing media.
   Note: We recommend an additional wash when retrieving cells from 4.8 kPa matrices.
8. Wash each well twice with 150 µL of 1x DPBS and combine with the cell suspension in Step 7.
   Note: We recommend an additional wash when retrieving cells from 4.8 kPa matrices.
   Note: We recommend visually inspecting the wells under the microscope to confirm that all cellular material has been retrieved. If there is remaining cellular material, repeat Step 8 until most or all of the cellular material has been retrieved.

9. For cell retrieval as:
    a. Intact spheroids/aggregates:
        i. Pellet cells from Step 8 at RT using cell-specific centrifugation conditions.
        ii. Aspirate and discard the supernatant.
        iii. Using a P1000 pipette, resuspend the cell pellet with 400 µL of 1x DPBS and gently pass through a 200 µm cell strainer into a microcentrifuge tube.
        Note: We recommend using a cell strainer pore size that will allow for the trapping of large gel fragments on the top of the cell strainer and permit cell spheroids or aggregates to pass through.
        iv. Collect the filtrate and proceed to Step 10.

    b. Single cells:
        i. Pellet cells from Step 8 at RT using cell-specific centrifugation conditions.
        ii. Aspirate and discard the supernatant.
        iii. Wash cells with 400 µL of 1x DPBS and pellet at RT using cell-specific centrifugation conditions
        iv. Aspirate and discard the supernatant.
        v. Resuspend the cell pellet in 400 µL of TrypLE Express and incubate in a 37°C water bath for 5-7 min.
        Note: TrypLE Express may be substituted with alternative enzymatic digestion reagents such as Trypsin/EDTA or Accutase. Incubation conditions may vary accordingly.
        vi. To deactivate the TrypLE, add 500 µL of complete media to the microcentrifuge tube/s. Gently pipette up and down several times to dissociate the spheroids into small aggregates.
        Note: It is important to deactivate the TrypLE before dissociating the cell spheroids/aggregates into single cells as this improves the recovery of viable cells.
        vii. Pellet the cells at RT using cell-specific centrifugation conditions.
        viii. Aspirate and discard the supernatant.
        ix. Resuspend the cell pellet in 400 µL of 1x DPBS (or 0.1% BSA) and pass the cell suspension through a small cell strainer (40-70 µm pore size) into a microcentrifuge tube.
        Note: If working with particularly adhesive cell types, repeating the cell straining step can improve the quality of the single-cell suspension. Remember to use a cell strainer with a pore size that is reflective of the size of the cultured cells. A second cell retrieval step may reduce cell yield.
10. Proceed with downstream analysis as required.

INVENTIA

Inventia Life Science Operations Pty Ltd
ABN 19 613 078 710
20-22 William Street,
Alexandria, NSW, 2015, AUSTRALIA

info@inventia.life | www.inventia.life