PROTOCOL

Cell Retrieval from RASTRUM™ Matrices

Introduction

RASTRUM Cell Retrieval Solution (CRS) is an enzymatic reagent that enables the rapid recovery of encapsulated cells from RASTRUM 3D cell models. This protocol describes a simple method to obtain live single cells or intact spheroids/aggregates, which may be used in downstream analysis methods, such as flow cytometry.

Storage and handling of CRS

Store CRS at -20°C upon receipt. For best results, do not leave CRS at room temperature (RT) for longer than 1 hour prior to use.

Note: CRS may be freeze-thawed up to three times without loss in activity. Unused diluted CRS should be discarded and not re-frozen for later use. Batch-to-batch variation in the colour of CRS is normal.

Equipment and reagents required, but not provided

- RASTRUM Cell Retrieval Solution (CA0011)
- 1x Dulbecco's Phosphate-Buffered Saline (DPBS) solution (ThermoFisher, 14190144 or similar)
- Serum-containing cell culture media
- Cell dissociation reagent e.g. TrypLE™ Express Enzyme (ThermoFisher, 12604013 or similar)
- 200 µm (Step 10a) and/or 40–70 µm (Step 10b) cell strainers (e.g. pluriStrainer Mini, 43-10040-50 and 43-10200-50 or similar)
- 1.5 mL microcentrifuge tubes
- 15 mL and/or 50 mL centrifuge tubes depending on well pooling strategy
- Single channel and multi-channel pipettes
- Optional: wide-bore pipette tips in all steps where intact spheroids are desired

Protocol

1. Thaw required volume of CRS (see Table 1) for 30 min at RT. Do not thaw at 37°C.

Table 1. Required CRS volumes for RASTRUM architectures.

Cell model architecture	Matrix stiffness (kPa)	CRS dilution	CRS volume per well (µL)	Incubation time (min)
Imaging Model	0.7, 1.1, 3.0	1:5*	75*	30
Imaging Model	4.8	No dilution	75	40
Large Plug Model	0.7, 1.1	No dilution	75	30
Large Plug Model	3.0, 4.8	No dilution	150	40
High-throughput Model	0.7, 1.1	No dilution	40	30

*Mix 1 part CRS with 4 parts PBS

2. Aspirate and discard media from wells by tilting plate at an angle to maximise fluid removal (Figure 1).

   Caution: Ensure your pipette tip does not pierce the cell model or inert base.

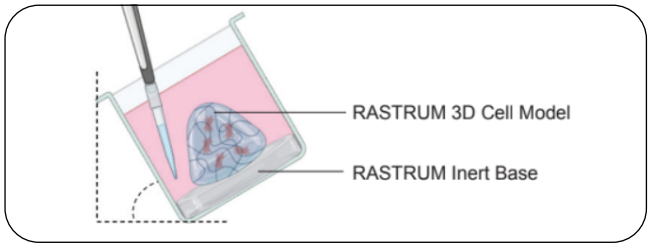

Figure 1. Pipetting with RASTRUM 3D cell models. The plate is tilted at an angle, and the pipette tip is positioned above the surface of the inert base.

3. Add prewarmed 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.

6. When CRS incubation is complete, add serum-containing media to each well to quench the enzymatic reaction:
   - For 96-well plates, make up to 200 µL.
   - For 384-well plates, make up to 60 µL.
7. *Gently* pipette the cell solution up and down 3–4 times while moving the pipette tip in a circular motion around the well. This will ensure that any remaining loose matrix is dissolved and all the cells are released into the well.

   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.
8. Transfer the cell suspension into a microcentrifuge or 15/50 mL tube containing media. We recommend a volume of 300 µL media per well. If pooling multiple wells, add 300 µL for every well pooled.

   Note: If combining multiple wells into a single sample, use a multi-channel pipette for Steps 7 and 8.
9. Wash each well with 100 µL of serum-containing media and combine with the cell suspension in Step 8.

   Note: We recommend inspecting the wells under the microscope to confirm that a sufficient number of cells have been retrieved. If required, repeat Step 9 up to three times (Figure 2).

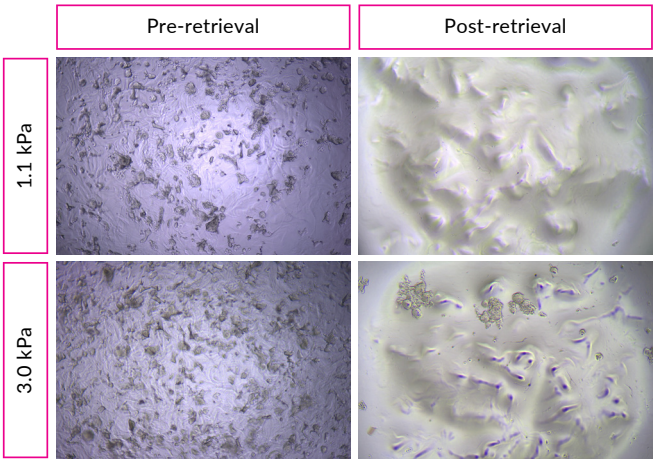

Figure 2. Representative brightfield images of RASTRUM Large Plug Model before and after incubation with CRS and following DPBS washes. Depending on the matrix, several DPBS washes may be required for efficient cell recovery.

Note: Cell retrieval yield from 4.8 kPa matrices is typically lower than other matrix classes. Consider pooling extra wells when using this matrix class.

10. To recover intact spheroids/aggregates, proceed with Step 10a. For recovery of single cells, proceed with Step 10b.

10a. Intact spheroids/aggregates

i. Pellet collected cells from Step 9 using cell-specific centrifugation conditions.

ii. Aspirate and discard the supernatant.

iii. Using a P1000 pipette with wide-bore tip, resuspend the cell pellet with 1x DPBS (500 µL for a microcentrifuge tube and 1 mL for a 15/50 mL tube) and gently pass through a wetted 200 µm cell strainer into a fresh tube. Wash the original tube with an additional 500 µL of 1x DPBS and pass through the strainer to maximise cell recovery.

iv. Collect the filtrate and proceed with downstream analysis.

10b. Single cells

i. Pellet cells from Step 9 using cell-specific centrifugation conditions.

ii. Aspirate and discard the supernatant.

iii. Wash cells with 1x DPBS (500 µL for a microcentrifuge tube and 1 mL for a 15/50 mL tube) and pellet using cell-specific centrifugation conditions.

iv. Aspirate and discard the supernatant.

v. Resuspend the cell pellet in 400 µL of TrypLE Express and incubate at 37°C for 5–7 min. Increase TrypLE volume if pooling many wells or cell pellet is large.

   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 serum-containing media to the tube(s). Gently pipette up and down several times to dissociate the spheroids. Increase media volume if a larger volume of TrypLE is used in the previous step.

vii. Pellet the cells using cell-specific centrifugation conditions.

viii. Aspirate and discard the supernatant.

ix. Resuspend the cell pellet in appropriate volume of 1x DPBS or serum-containing media depending on the downstream application (we recommend 500 µL for a microcentrifuge tube and 1 mL for a 15/50 mL tube).

x. Pass the cell suspension through a wetted small cell strainer (40–70 µm pore size) into a 1.5 mL microcentrifuge or 15/50 mL tube.

xi. Collect the filtrate and proceed with downstream analysis.

FAQs

1. **Will CRS dissolve the inert base?**

   No, CRS will dissociate RASTRUM 3D cell models, but the inert base will remain intact.

2. **What factors might impair cell retrieval?**

   Piercing the inert base with the pipette tip may create matrix fragments that could interfere with cell pelleting and decrease cell yield. Additionally, significant deposition of extracellular matrix components by cells may reduce cell retrieval efficiency.

3. **Can I extract RNA or protein from retrieved cells using this protocol?**

   Yes, lysis buffer may be added to the retrieved cell pellet. For RT-qPCR and RNA sequencing applications, we recommend following our direct RNA Extraction Protocol, which does not require cell retrieval.

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Document ID SUP.KIT.0001.PRO_Rev02

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