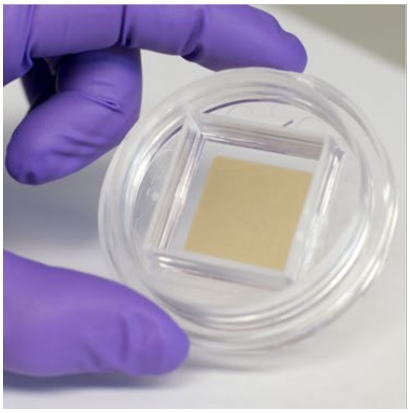
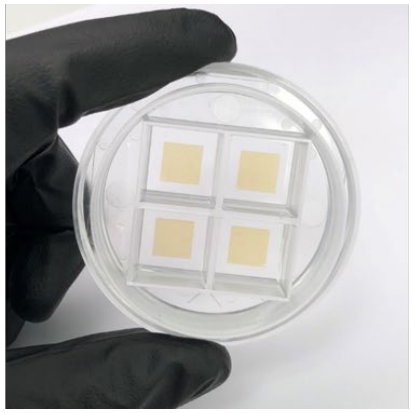
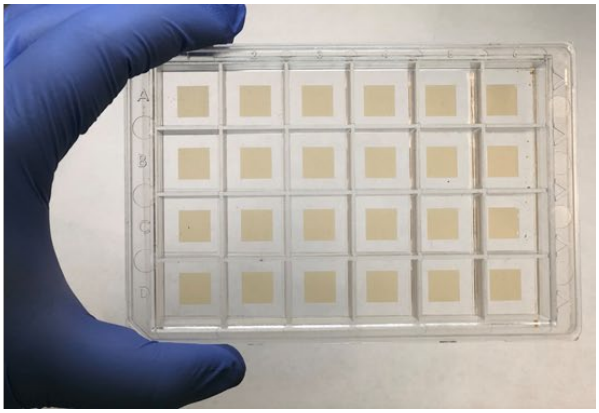
CELL Microsystems®

CellRaft® Array

Microwell Array with Releasable CellRafts for Cell Isolation

Single cell screening, isolation, and retrieval using CellRaft Technology

For use with the CellRaft AIR® System and CellRaft System for Inverted Microscopes

User Manual

Table of Contents

Notices

Cell Microsystems, Inc.
801 Capitola Dr.
Suite 10
Durham
North Carolina, 27713
USA

Contact Information

Cell Microsystems, Inc.
801 Capitola Dr.
Suite 10
Durham
North Carolina, 27713
USA

Internet: http://www.cellmicrosystems.com
Phone: 252-285-9842

To purchase CellRaft Arrays or other Cell Microsystems products, contact sales@cellmicrosystems.com.

For technical questions and other support, contact support@cellmicrosystems.com.

For general information about Cell Microsystems, contact info@cellmicrosystems.com.

Document Conventions

This icon calls attention to important safety notes

Warning! A warning indicates the potential for bodily harm and tells you how to avoid the problem.

Caution A caution indicates potential damage to the product and tells you how to avoid the problem.

Note: **Bold** text is primarily used for **emphasis**.

Revision History

Document Number: PD-015

Revision	Effective Date	Changes
00	14 JAN 2019	Initial Release
01	11 NOV 2019	Added details pertaining to CellRaft HexaQuad Array
02	04 SEP 2020	Clarified and added user instructions across all array formats
03	01 JUN 2021	Added details pertaining to crystallization of anti-bubble coating
04	20 SEP 2022	Changed all references from CytoSort to CellRaft Arrays

1. Intended Use Statement

CellRaft Arrays are intended for use with other Cell Microsystems products, including the CellRaft Automated Isolation and Retrieval (AIR) System and the CellRaft System for Inverted Microscopes. The CellRaft Array is a single-use consumable used to image cells seeded on the array for subsequent isolation and retrieval using a magnetic wand. **The system is not intended for use in diagnostic or other clinical applications.**

For Research Use Only.
Not for use in diagnostic procedures.

2. Warnings and Precautions

- **For Research Use Only. Not for use in diagnostic procedures.**
- Use standard lab precautions when handling and using the product.
- Physiological samples of all types from any source should be treated as biohazardous according to your organization's approved procedures.
- Performance can be adversely affected by extraneous contaminants such as dust, dirt, adhesive, etc. on the CellRaft Array. Make sure CellRaft Arrays are clean and free of debris before use.

3. Limited Warranty and Liability

The CellRaft Arrays sold by Cell Microsystems have been designed and tested to perform according to the published specifications. Cell Microsystems warrants that these products will conform to those specifications and be free from defects in workmanship and materials for a period of twelve (12) months from the date of shipment. Defective products will be repaired or replaced, at Cell Microsystems option, during this period provided the purchaser has used the products under conditions of normal and proper use, but not for damage caused by the purchaser.

THE FOREGOING WARRANTY IS THE SOLE AND EXCLUSIVE WARRANTY MADE BY CELL MICROSYSTEMS. CELL MICROSYSTEMS MAKES NO OTHER WARRANTY OR GUARANTEE OF ANY KIND, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION IMPLIED WARRANTIES OF FITNESS FOR A PARTICULAR PURPOSE OR MERCHANTABILITY. CELL MICROSYSTEMS' TOTAL LIABILITY AND PURCHASERS EXCLUSVIE REMEDY FOR ANY CLAIM OR LIABILITY ASSOCIATED WITH THE PRODUCTS SOLD, WHETHER BASED IN TORT, CONTRACT, STRICT LIABILITY OR ANY OTHER LEGAL THEORY IS EXPRESSLY LIMITED TO, AT CELL MICROSYSTEMS OPTION, REPLACEMENT, REPAIR, OR REWORK, AS APPLICABLE, OF NONCONFORMING PRODUCT OR PAYMENT IN AN AMOUNT NOT TO EXCEED, IN THE AGGREGATE, THE PURCHASE PRICE OF THE SPECIFIC PRODUCT FOR WHICH DAMAGES ARE CLAIMED. IN NO EVENT SHALL CELL MICROSYSTEMS BE LIABLE FOR ANY OTHER DAMAGES, LOSSES OR EXPENSES, INCLUDING, WITHOUT LIMITATION, INDIRECT, INCIDENTAL, PUNITIVE, CONSEQUENTIAL, EXEMPLARY, OR SPECIAL DAMAGES.

4. Quick Start Guide for the CellRaft Array

NOTE: The CellRaft Array should be prepared in accordance with standard aseptic technique in a sterile biosafety cabinet or environment.

1. Pre-warm sterile DPBS and the appropriate cell culture media to 37°C.
2. Remove CellRaft Array from sterile packaging. Add the appropriate volume (Table 1) of pre-warmed DPBS to each reservoir for 3 minutes. Aspirate the liquid and repeat the wash procedure twice more. **Do not let the CellRaft Array dry completely at any point during the preparation process**.
    a. Note: Prepared arrays may be stored at 4°C in DPBS.
3. If desired, remove DPBS wash and replace with the appropriate volume of pre-warmed media (Table 1) or proceed directly to cell seeding.
    a. If the seeding insert will be used for cell seeding, place insert into the reservoir after DPBS is aspirated and prior to adding fresh media.

Table 1

CellRaft Array Format	Catalog Number(s)	Wash Volume per Reservoir
Single Reservoir	CS100S CS200S	3mL
Quad Reservoir HexaQuad	CS100Q CS200Q CS100HQ CS200HQ	1mL

4. Prepare the appropriate concentration of dilute cell suspension for seeding the array. To maximize the number of single cells on the CellRaft Array, it is recommended to seed a total number of cells equal to approximately ⅓ to ½ the number of microwells present in each reservoir (Table 2).
    a. Note: The optimal number of cells per reservoir will be application and cell line dependent, and as many or as few cells as desired can be seeded.

Table 2

CellRaft Array Format	Catalog Number	Microwells per Reservoir	Recommended Cells per Reservoir	Cell Seeding Volume per Reservoir	
				No Insert	Insert
100μm Single	CS100S	40,000	$1.0x10^4$ - $2.0x10^4$	5mL	4mL
200μm Single	CS200S	10,000	$3.0x10^3$ - $5.0x10^3$	5mL	4mL
100μm Quad 100μm HexaQuad	CS100Q CS100HQ	6,400	$2.0x10^3$ - $3.2x10^3$	1mL	0.5mL
200μm Quad 200μm HexaQuad	CS200Q CS200HQ	1,600	$0.5x10^3$ - $0.8x10^3$	1mL	0.5mL

5. Aspirate media from the array and seed the appropriate volume of cells per reservoir (Table 2) dropwise in the center of each CellRaft Array reservoir. Incubate the array and allow cells to settle and adhere. Time for this step will be dependent upon cell type and culture conditions, and cells can be imaged (either on the CellRaft AIR System or benchtop microscope) to confirm they have settled and adhered to the CellRaft Array.
    a. Note: For quad and HexaQuad CellRaft Arrays, do not tip the plate after addition of cell suspension to ensure cells settle appropriately in the microwells.
6. If the CellRaft Seeding Insert was used, after cells have adhered (no less than 2 hours), remove the insert and increase the volume of media to 5 mL for the single reservoir array or 1 mL per reservoir for the quad reservoir and HexaQuad arrays.

5. Features of the CellRaft Array

5.1. The CellRaft Array

The CellRaft Array comprises an elastomeric (polydimethylsiloxane, PDMS) microwell array filled with individually releasable polystyrene elements called CellRafts (**Figure 1A**). The polystyrene CellRafts are loaded with iron oxide nanoparticles, facilitating isolation and collection with a magnetic wand. The array is housed in a petri dish-sized 65 mm round cassette or SLAS-format cassette with walls surrounding each microwell array to create a reservoir for media, buffer, or other reagents (**Figure 1B**). Cells are seeded on the CellRaft Array at a recommended density to ensure a high percentage of cells reside as a single cell in a microwell (**Figure 1C**).

When used with the CellRaft AIR System, automated detection of single cells can be accomplished using brightfield or fluorescent microscopy. The AIR System also allows users to scan the array to identify individual cells of interest in either User Navigation and Selection Mode or by performing a Full Array Scan. A list of array locations (i.e., individual CellRaft addresses) can also be determined during off-instrument imaging, and this list can be imported as a spreadsheet for subsequent isolation of CellRafts.

The CellRaft Array can be surface-coated similar to any plastic cell culture consumable with extracellular matrix proteins or non-specific biological adhesives. See Section 6.5 for more information regarding surface coating protocols.

NOTE: The CellRaft Array is a single-use consumable. Re-use of a CellRaft Array compromises the efficiency of cell seeding and adherence, as well as release and retrieval of CellRafts and is not recommended or supported as a use case.

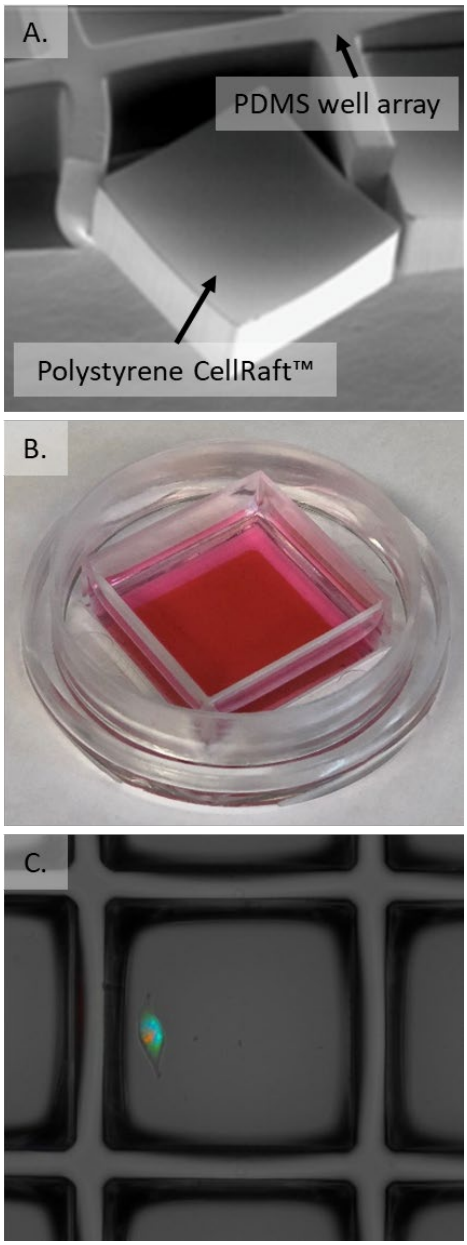


Figure 1. The CellRaft Array comprises a PDMS well array and polystyrene CellRafts (A). Cells are seeded on the array for imaging and isolation in a contiguous volume of media (B). Single cell on a CellRaft (C).

5.2. CellRaft Addresses Within the CellRaft Array

Each CellRaft within the CellRaft Array has a unique address describing its row and column position within the array (**Figure 2**). For the 65-mm single reservoir and quad reservoir arrays, each lettered row and column consist of 10 individual CellRafts which begin their numbering at 0 and end at 9 (i.e., CellRafts in the "A" Row comprise A0 through A9). Only the 0-designated CellRaft (i.e., A0A0) is labeled with its specific address. The 5th CellRaft in a given row or column is labeled with a small dot in the center of the CellRaft. This is depicted in more detail in **Figure 3.**

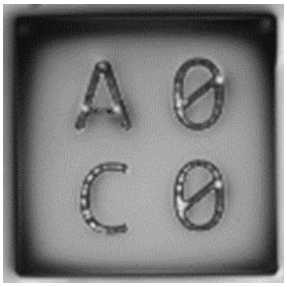


Figure 2: CellRaft addresses represent a row and column position within the CellRaft Array. This CellRaft is in row A and column C.

Given the large number of CellRafts in the CellRaft HexaQuad Array, the alphanumeric row and column addresses have been replaced by three-digit decimal numbers. For a 100µm HexaQuad Array, the rows begin their numbering at 000 in reservoirs A6 – D6 and end at 479 in reservoirs A1 – D1, while the columns begin at 000 in reservoirs A1 – A6 and end at 319 in reservoirs D1 – D6 (see **Figure 4**). For a 200µm HexaQuad Array, the rows extend from 000 to 239 and the columns from 000 to 159. Every 5th CellRaft in a given row or column is labeled with a small dot in the center of the CellRaft.

The CellRaft Array can be imaged on a standard inverted microscope and the user can visually identify raft addresses to manually generate a list of addresses in a Microsoft Excel® spreadsheet. The spreadsheet can be imported directly into the AIR System software. For more information on use with the AIR System software, please refer to the AIR System User Manual.

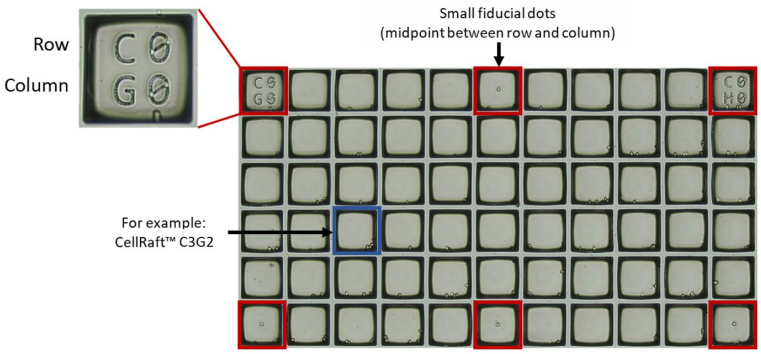


Figure 3: Alphanumeric CellRaft address system for single and quad reservoir CellRaft Arrays. C0G0 is in the upper left corner. This represents the CellRaft in Row C0 and Column G0. Every 5th row or column is marked by a small fiducial dot on the CellRaft.

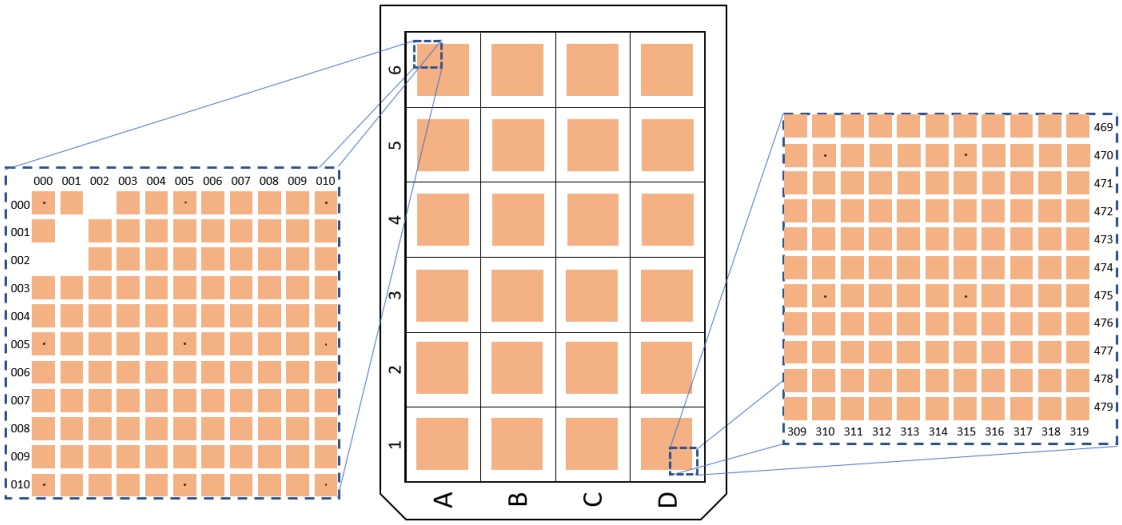


Figure 4: Three-digit decimal CellRaft address system for CellRaft HexaQuad Arrays (100µm example shown). CellRafts are numbered based on array orientation within the CellRaft AIR System, with CellRaft [000, 000] in the upper-left corner of reservoir A6 and CellRaft [479, 319] in the lower-right corner of reservoir D1. Every 5th row or column is marked by a small fiducial dot on the CellRaft™.

Preparing the CellRaft Array

6.1. Storage

Prior to use, CellRaft Arrays should be stored in their packages at room temperature in a dry location.

6.2. Wetting the CellRaft Array Before Use

The CellRaft Array is covered with a proprietary water-soluble biocompatible coating which prevents air bubble formation when liquid is added to the array. A simple wash procedure is required to completely remove the coating immediately prior to cell seeding on the CellRaft Array (**Figure 5**).

To remove the anti-bubble coating and prepare the CellRaft Array for cell seeding:

1. Warm buffer (such as phosphate buffered saline, PBS) or media to approximately 37°C.
2. Add the appropriate volume of buffer or media to each reservoir of the array (Table 1).
3. Incubate at room temperature for 3 minutes.
4. Aspirate the buffer or media.
5. Repeat this wash protocol twice for a total of 3 washes.

NOTE: Once prepared, the CellRaft Array must be kept wet. Do not allow the CellRaft Array to become dry as bubbles may form in the microwells, which can negatively impact cell seeding, image quality, and CellRaft release efficiency.

NOTE: In some cases (e.g. coating during manufacturing, prolonged storage), the anti-bubble coating can crystalize and form white particulates that are visible on the array surface (arrows, Figure 6). This crystallization *does not* cause any loss of function, integrity, or sterility of the array and is not a cause for discontinuation of use. Proper preparation of the array using *warm* PBS as described above will dissolve and remove the white crystals prior to cell seeding.

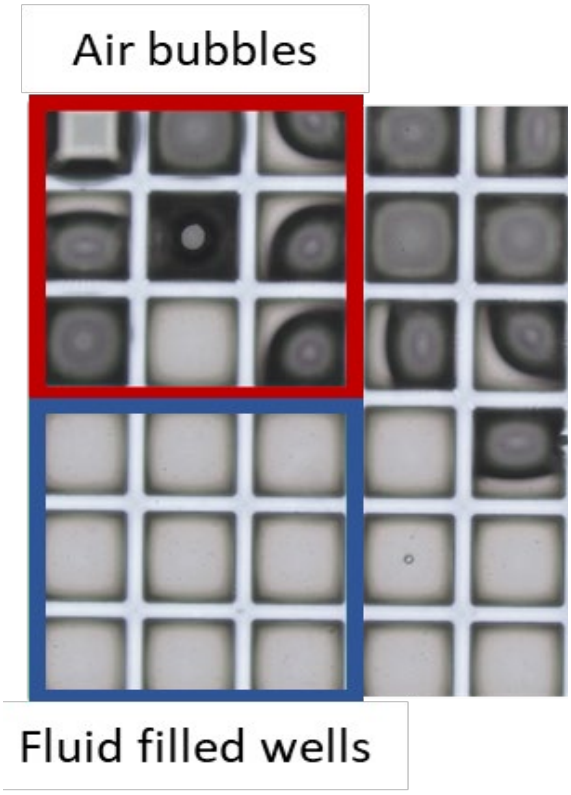


Figure 5. Air bubbles form when anti-bubble coating is not fully dissolved. Fluid fills wells after coating is removed completely.

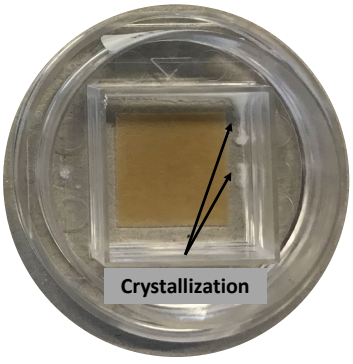


Figure 6. Crystallization of anti-bubble coating.

6.3. Seeding Cells on the CellRaft Array

Cells should be counted via hemocytometer or other cell counting method prior to seeding on the CellRaft Array. The cell distribution on the CellRaft Array roughly follows a Poisson distribution. To maximize the number of single cells on the array, it is recommended to seed a total number of cells equal to approximately ⅓ to ½ the number of microwells present in each reservoir of the CellRaft Array (Table 2).

Note: The appropriate concentration of cells required to ensure single cell seeding of a given cell type will require experimental determination. It is recommended to test a range of concentrations for each new cell type to ensure a sufficient number of single cells attaching per array.

Prepare the appropriate volume and concentration of cell suspension for seeding the array (Table 2).

Seed the cells dropwise in the center of each reservoir of the array. Incubate the CellRaft Array and allow cells to settle and adhere. Time for this step can vary from 30 minutes to several hours and will be dependent upon cell type and culture conditions; cells can be imaged (either on the CellRaft AIR System or benchtop microscope) to confirm they have settled and adhered to the CellRaft Array.

Note: For HexaQuad Arrays, it is recommended to work quickly to seed cells after aspirating the final wash. If cell seeding will take longer than 5 minutes, aspirate as few wells at a time as necessary to minimize drying of the reservoirs during cell seeding. It is also recommended that the plate not be tilted once cells have been added, in order to allow the cells to settle evenly within the array.

6.4. Seeding Cells using the CellRaft Seeding Insert

Samples with very few cells (<500) may require the use of the CellRaft seeding insert to increase the percentage of microwells containing a single cell. The CellRaft seeding insert can also be used with samples of any cell titer to increase the overall number of single cells available for isolation (**Table 3**).

If the CellRaft seeding insert (**Figure 7**) is to be used, place the insert in the CellRaft Array after the array preparation washes (see Section 0), prior to cell seeding. The cell suspension should be added dropwise to the center of each reservoir in the array in 4 mL total volume for the single reservoir array or 500 µL per reservoir for the quad reservoir and HexaQuad arrays (Table 2).

Total # Cells Seeded	Total # Single Cells		% Single Cells
500	No insert	110	22%
	Insert	393	79%
3,000	No insert	710	24%
	Insert	1692	56%
10,000	No insert	1606	16%
	Insert	2602	26%

Table 3. Cell seeding data with and without the use of the CellRaft seeding insert for a 200µm single-reservoir array. Percentage of single cells refers to the percentage of cells seeded that are residing as a single cell in a microwell on the CellRaft Array.

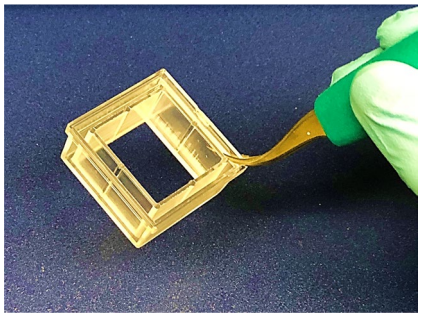


Figure 7. The CellRaft seeding insert can be used to increase the percentage of single cells, especially for samples with a low cell titer.

NOTE: Cell seeding insert must be removed prior to use with the CellRaft AIR System. After the insert is removed, media should be added to the CellRaft Array to bring the total volume in the reservoir to 5 mL for the single reservoir array or 1 mL per reservoir for the quad reservoir and HexaQuad arrays.

Additional details regarding use of the CellRaft Array seeding insert can be found in the RaftNotes section of the Cell Microsystems website.

6.5. Applying Coatings to the CellRaft Array

The CellRaft Array contains materials (PDMS and polystyrene) that are commonly found in tissue culture vessels (e.g., Petri dish, culture flask, multi-well plate, etc.) and are compatible with adherent cells without the requirement for a coating. However, if required for a given cell type, the arrays can be coated using standard protocols provided by the coating manufacturers. For non-adherent cells, a cell culture coating can be applied to the CellRaft Array to ensure adherence of suspension cells.

Table 4 summarizes the cell culture coatings that have been specifically tested with the CellRaft Array and gives recommended conditions for each coating. These protocols are intended only as a basic guideline; coatings should be optimized with specific cell lines and culture conditions before use with the CellRaft Array. Coatings should be applied after the array has been prepared (see Section 6.2) and prior to seeding cells on the array.

Coating	Manufacturer	Recommended concentration	Incubation	Wash steps
Poly-L-lysine	Sigma	1.5 mL of 0.01% solution	1 hour, 37˚C	Sterile water (x1)
Cell-Tak	Corning	31.5 ug/mL (in PBS)	1 hour, 37˚C	Sterile water (x3)
Fibronectin	Corning	10 ug/mL (in PBS)	1 hour, 37˚C	PBS (x2)
Gelatin	Sigma	1 mL of 2% solution	1 hour, 37˚C	None
RGD peptides	Selleckchem	10 ug/mL (in PBS)	1 hour, 37˚C	Sterile water (x1)
Matrigel	Corning	1 mL of 1% solution	1 hour, 37˚C	PBS (x2)
Collagen type IV	Sigma	10 ug/mL (in PBS)	1 hour, 37˚C	PBS (x2)

Table 4. Coating information and application conditions for use with CellRaft Arrays.

NOTE: During the application of cell culture coatings, the CellRaft Array must be kept wet. Do not allow the CellRaft Array to become dry as bubbles may form in the microwells, which can negatively impact cell seeding, image quality, and CellRaft release efficiency. Once coated, prepared arrays can be stored in DPBS at 4C for at least one week.

Additional details regarding use of the coatings on the CellRaft Arrays can be found in the RaftNotes section of the Cell Microsystems website.

6.6. Staining Cells for Imaging on the CellRaft Array

Many vital dyes and fluorophores are compatible with the CellRaft Array. Cells can be stained either in suspension prior to seeding, or after settling and adhering to the CellRafts. Several red dyes have been found to increase background fluorescence when stained on-array and should be used for suspension staining only. Some general recommendations are made in **Table 5**. Dye concentrations and incubation times should be optimized for individual cell types and cell culture conditions. An example of a triple-stained NIH/3T3 (ATCC® CRL-1658™) mouse cell is shown in **Figure 8**.

Additional details regarding staining cells on the CellRaft Array for use with the CellRaft AIR System can be found in the RaftNotes section of the Cell Microsystems website.

6.7. Imaging Cells on the CellRaft Array

Most microscopy methods are compatible with the CellRaft Array; however, the release and isolation systems provided by Cell Microsystems may preclude some microscopy modes. The CellRaft System for Inverted Microscopes requires the use of an inverted microscope with an objective having at least 10 mm working distance, which can prevent the use of higher magnification (i.e., 20X, 40X, etc.) objectives.

The CellRaft AIR System employs a 10X objective and generates brightfield and fluorescence images in 3 channels (for information on specific fluorescent channels and excitation/emission spectra, please refer to the AIR System User Manual).

If different magnifications and/or microscopy modes are needed for a given experiment, it is recommended to image cells on the CellRaft Array using a microscope that supports the desired imaging settings. The user can then record the CellRaft addresses for cells of interest and release those CellRafts on either the CellRaft System for Inverted Microscopes or the automated CellRaft AIR System. Please see Section 5.2 for more details on the CellRaft Array address system.

For guidance on CellRaft Array compatibility with various microscopy methods, contact Cell Microsystems, Inc. using the contact information on page 3 of this manual.

Dye	Concentration	Time
CellTracker™ Green CMFDA	5 µM	30 min
CellTracker™ Blue	5 µM	30 min
CellTracker™ Red	5 µM	30 min
SYTO® 13 Green	5 µM	30 min
SYTO® 41 Blue	5 µM	30 min
SYTO® 17 Red	5 µM	30 min
Hoechst 33342	1.6 µM (1 µg/mL)	30 min
MitoTracker® Red FM	500 nM	30 min

Table 5. Vital dyes and recommended conditions for use with CellRaft Arrays. Dye concentrations reflect the final concentration used on-array or in suspension.

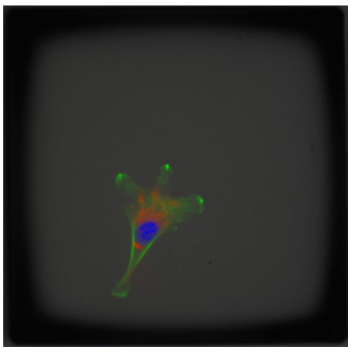


Figure 8. Example of a NIH/3T3 mouse cell stained with Alexa Fluor 488® Phalloidin (actin), MitoTracker® Red FM (mitochondria), and Hoechst 33342 (nucleus).

6.8. Preparing the CellRaft Array for Isolation

Although the cells can be cultured in the CellRaft Array in any type (including serum free) and volume of desired media (at least 300uL per quad/HexaQuad and 1mL per single reservoir) during the growth and imaging time period, it is imperative that there is an appropriate volume of culture media containing at least 10% protein (e.g. FBS, BSA, or other appropriate cell culture grade serum) in order for isolation to occur with high efficiency. Immediately prior to any isolation, ensure that the fluid volume in the reservoir is at least 1mL per quad reservoir (quad or HexaQuad) or 5mL per single-reservoir array. It is recommended for all array types that all media is aspirated and fresh media is added to the reservoirs immediately prior to beginning isolation.

Note: Serum containing media is not required in the collection plates for deposit to occur.

7. Troubleshooting

7.1. Bubbles Forming in the Microwells of the CellRaft Array

If large bubbles are observed in the microwells of the CellRaft Array, this is likely because the anti-bubble coating was not fully dissolved. If bubbles are observed prior to cell seeding, repeat the wash protocol described in Section 0 to ensure full dissolution of the coating.

If bubbles are observed after cells have been seeded on the CellRaft Array, slight agitation of the array may help gently dissolve the remaining anti-bubble coating without harming the seeded cells.

7.2. Fewer Single Cells than Expected

It is highly recommended to optimize the cell seeding concentration for each cell line prior to beginning an experiment. Some cell lines may require higher cell:microwell ratios (i.e 1:1 or 2:1) than typically suggested. For example, testing four different cell concentrations in a quad array is an efficient and effective way to determine the optimal cell seeding density.

7.3. Lower Collection Efficiency than Expected

Ensure that there is an appropriate volume (see Section 6.8) of 10% serum containing media in each reservoir being isolated to ensure maximal collection efficiency.

For additional troubleshooting or technical support questions, contact Cell Microsystems Technical Support at support@cellmicrosystems.com.


CELL
Microsystems®