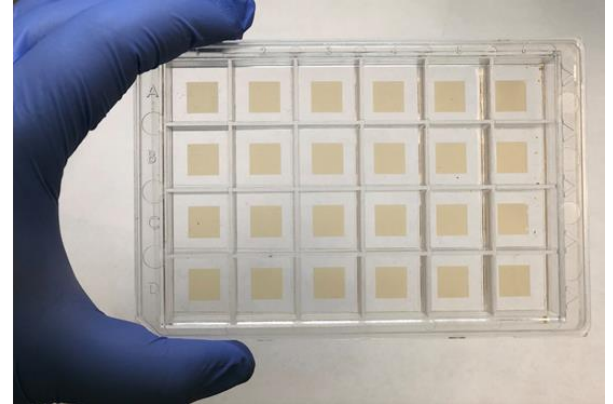
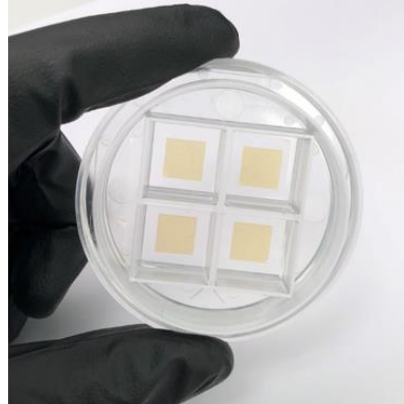
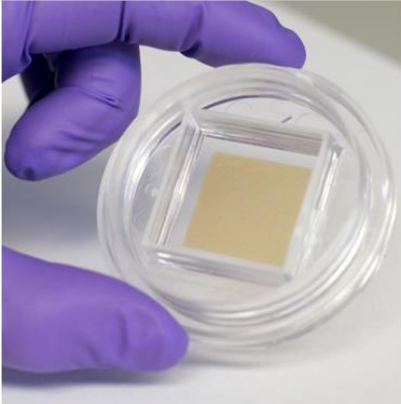


CELL
Microsystems



CytoSort™ 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

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Notices

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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 CytoSort HexaQuad Array
02	04 SEP 2020	Clarified and added user instructions across all array formats

1. Intended Use Statement

CytoSort™ 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 CytoSort™ 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 CytoSort Array. Make sure CytoSort Arrays are clean and free of debris before use.

3. Limited Warranty and Liability

The CytoSort 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 EXCLUSIVE 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 CytoSort™ Array

NOTE: The CytoSort™ 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 CytoSort™ 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 CytoSort™ 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

CytoSort 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 CytoSort™ Array, it is recommended to seed a total number of cells equal to approximately $\frac{1}{3}$ to $\frac{1}{2}$ 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

CytoSort Array Format	Catalog Number	Microwells per Reservoir	Recommended Cells per Reservoir	Cell Seeding Volume per Reservoir	
				No Insert	Insert
100µm Single	CS100S	40,000	$1.0 \times 10^4 - 2.0 \times 10^4$	5mL	4mL
200µm Single	CS200S	10,000	$3.0 \times 10^3 - 5.0 \times 10^3$	5mL	4mL
100µm Quad 100µm HexaQuad	CS100Q CS100HQ	6,400	$2.0 \times 10^3 - 3.2 \times 10^3$	1mL	0.5mL
200µm Quad 200µm HexaQuad	CS200Q CS200HQ	1,600	$0.5 \times 10^3 - 0.8 \times 10^3$	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 CytoSort™ 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 CytoSort™ Array.
 - a. Note: For quad and HexaQuad CytoSort™ 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 CytoSort™ Array

5.1. The CytoSort™ Array

The CytoSort™ 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 CytoSort™ 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 CytoSort™ 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 CytoSort™ Array is a single-use consumable. Re-use of a CytoSort™ 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.

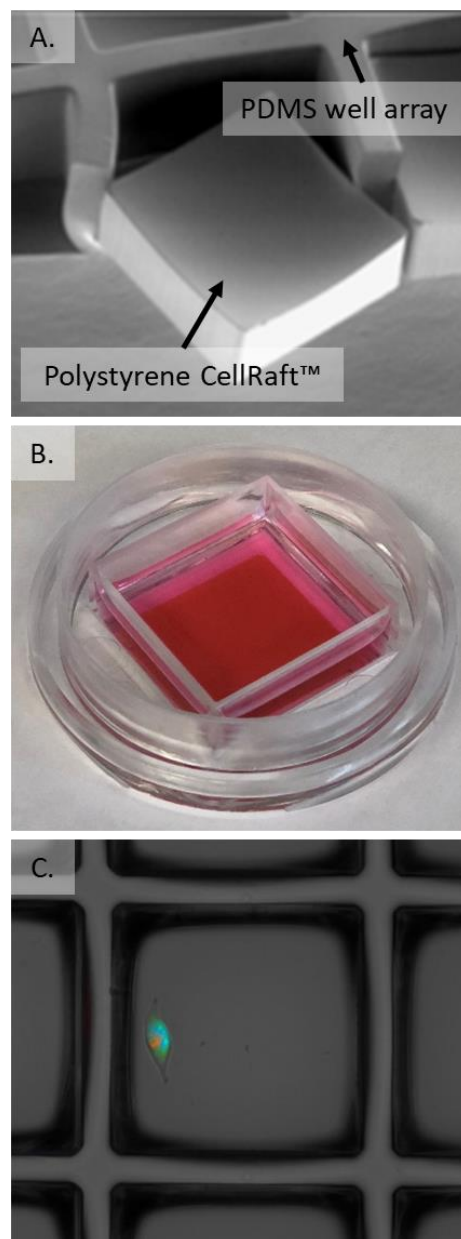


Figure 1. The CytoSort™ Array comprises a PDMS well array and polystyrene CellRafts™ (A). Cells are seeded on the array for imaging and isolation in a contiguous volume of media (B). Single cell on a CellRaft™ (C).

5.2. CellRaft™ Addresses Within the CytoSort™ Array

Each CellRaft™ within the CytoSort™ 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**.

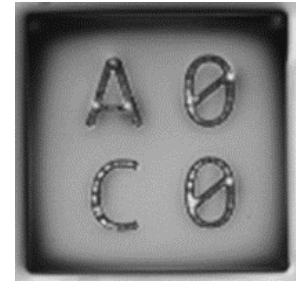


Figure 2: CellRaft addresses represent a row and column position within the CytoSort™ Array. This CellRaft is in row A and column C.

Given the large number of CellRafts in the CytoSort 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™.

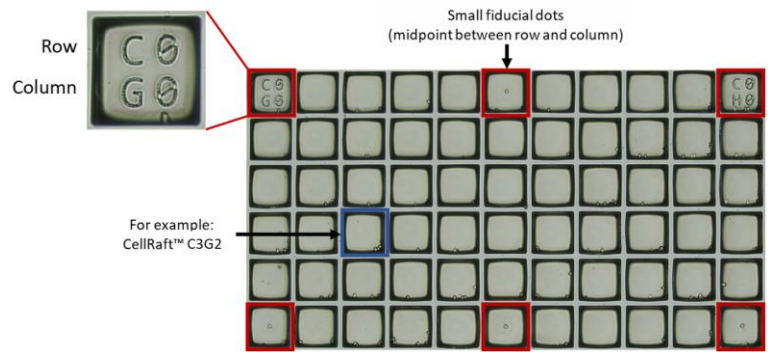


Figure 3: Alphanumeric CellRaft™ address system for single and quad reservoir CytoSort Arrays. C0G0 is in the upper left corner. This represents the CellRaft™ in Row C0 and Column G0. Every 5th row or column is marked by a small fiducial dot on the CellRaft™.

The CytoSort™ 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.

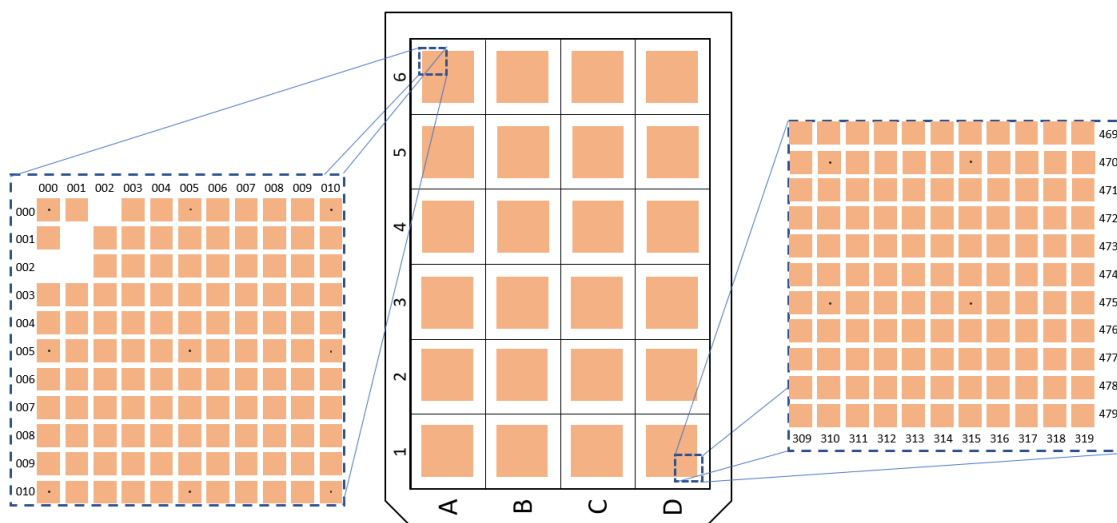


Figure 4: Three-digit decimal CellRaft™ address system for CytoSort HexaQuad Arrays (100µm example shown). CellRafts are numbered based on array orientation within the CellRaft AIR System, with CellRaft [000, 000] in the upper-left corner of reservoir A6 and CellRaft [479, 319] in the lower-right corner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

Preparing the CytoSort™ Array

6.1. Storage

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

6.2. Wetting the CytoSort™ Array Before Use

The CytoSort™ 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 CytoSort™ Array (**Figure 5**).

To remove the anti-bubble coating and prepare the CytoSort™ 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 CytoSort™ Array must be kept wet. Do not allow the CytoSort™ Array to become dry as bubbles may form in the microwells, which can negatively impact cell seeding, image quality, and CellRaft™ release efficiency.

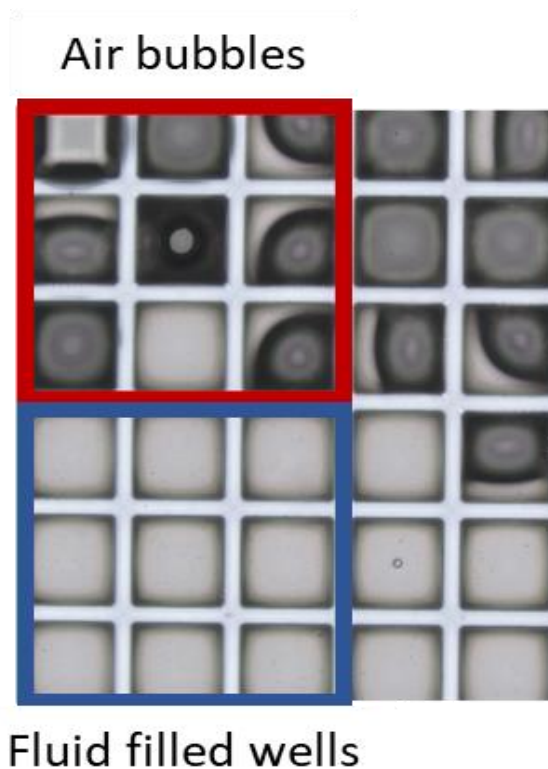


Figure 5. Air bubbles form when anti-bubble coating is not fully dissolved. Fluid fills wells after coating is removed completely.

6.3. Seeding Cells on the CytoSort™ Array

Cells should be counted via hemocytometer or other cell counting method prior to seeding on the CytoSort™ Array. The cell distribution on the CytoSort™ 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 $\frac{1}{3}$ to $\frac{1}{2}$ the number of microwells present in each reservoir of the CytoSort™ 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 CytoSort™ 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 CytoSort™ 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 6) is to be used, place the insert in the CytoSort™ 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 μ L 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 200 μ m 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 CytoSort™ Array.

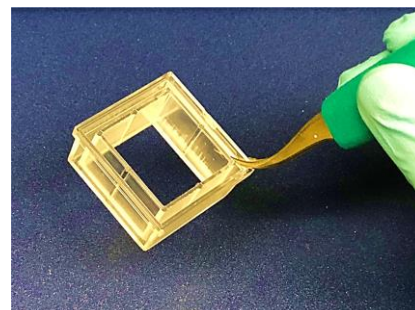


Figure 6. The CellRaft™ seeding insert can be used to increase the percentage of single cells, especially for samples with a low cell titer.

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 CytoSort™ 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 CytoSort Array seeding insert can be found in the RaftNotes section of the Cell Microsystems website.

6.5. Applying Coatings to the CytoSort™ Array

The CytoSort™ 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 CytoSort™ Array to ensure adherence of suspension cells.

Table 4 summarizes the cell culture coatings that have been specifically tested with the CytoSort™ 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 CytoSort™ 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 CytoSort™ Arrays.

NOTE: During the application of cell culture coatings, the CytoSort™ Array must be kept wet. Do not allow the CytoSort™ 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 CytoSort Arrays can be found in the RaftNotes section of the Cell Microsystems website.

6.6. Staining Cells for Imaging on the CytoSort™ Array

Many vital dyes and fluorophores are compatible with the CytoSort™ 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 7**.

Additional details regarding staining cells on the CytoSort 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 CytoSort™ Array

Most microscopy methods are compatible with the CytoSort™ 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 CytoSort™ 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 CytoSort™ Array address system.

For guidance on CytoSort 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 CytoSort™ Arrays. Dye concentrations reflect the final concentration used on-array or in suspension.

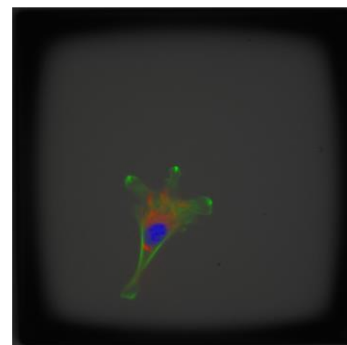


Figure 7. Example of a NIH/3T3 mouse cell stained with Alexa Fluor 488® Phalloidin (actin), MitoTracker® Red FM (mitochondria), and Hoechst 33342 (nucleus).

6.8. Preparing the CytoSort™ Array for Isolation

Although the cells can be cultured in the CytoSort 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 CytoSort™ Array

If large bubbles are observed in the microwells of the CytoSort™ 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 CytoSort™ Array, slight agitation of the array may help gently dissolve the remaining anti-bubble coating without harming the seeded cells.

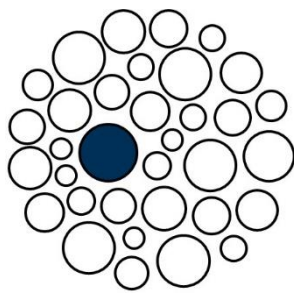
7.2. Fewer Single Cells than Expected

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.



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