Immunophenotyping Cancer Cells Using Flow Cytometry

Cancer Biology Applications on the BD Accuri™ C6

Features

- Immunophenotype cancer cells based on surface markers
- Screen cancer cells for expression of surface proteins

Characteristic	MDA-MB-231	MDA-MB-468	MCF-7
Tumor classification	Epithelial breast adenocarcinoma	Epithelial breast adenocarcinoma	Epithelial breast adenocarcinoma
Derivation	Metastatic site (pleural effusion)	Metastatic site (pleural effusion)	Metastatic site (pleural effusion)
Enrichment	Cancer stem cell phenotype ($CD44^+CD24^-$)	None	Small subpopulation ($CD44^+CD24^-$)

Table 1. Breast cancer cell lines used in immunophenotyping examples[1,2]

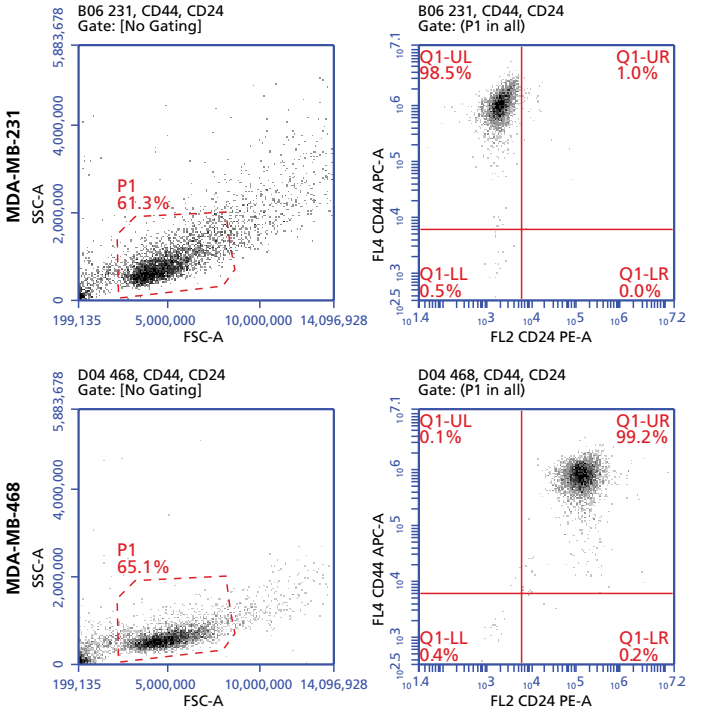


Figure 1. Immunophenotyping breast cancer cell lines for cancer stem cell markers

MDA-MB-231 and MDA-MB-468 cells (human epithelial breast adenocarcinoma; ATCC) were disassociated with BD™ Accutase™ Cell Detachment Solution (Cat. No. 561527) and stained with BD Pharmingen™ Mouse Anti-Human CD24 PE and BD Pharmingen™ Mouse Anti-Human CD44 APC (Cat. Nos. 555428 and 559942). Data was acquired on a BD Accuri C6 and analyzed using BD Accuri™ C6 software. **Results:** Cells were initially gated based on light scatter properties (left plots). As expected, MDA-MB-231 cells (upper plots) expressed a cancer stem cell phenotype ($CD44^+CD24^-$) while MDA-MB-468 cells (lower plots) expressed both CD24 and CD44. Gates were drawn based on isotype controls (data not shown).

A personal flow cytometer in the lab provides many advantages for cell and cancer biology studies. When cells are ready for analysis or rare tumor samples arrive, it's crucial to have a flow cytometer at hand, ready to go. This data sheet shows the kind of rich data you can generate using the BD Accuri™ C6 personal flow cytometer for two kinds of cancer biology studies.

Experiment 1: Immunophenotyping of cancer cell lines

Experiment 1 demonstrates immunophenotyping of MDA-MB-231 and MDA-MB-468, two of the breast cancer cell lines shown in Table 1. Immunophenotyping is one of the foremost applications of flow cytometry because of its ability to recognize different cell types based on the expression of surface and intracellular proteins.

Figure 1 shows the results when MDA-MB-231 and MDA-MB-468 cells were tested for expression of CD24 and CD44, two known cancer cell markers. With two lasers and four fluorescence detectors, the BD Accuri C6 could have tested for expression of two or more additional surface or intracellular markers as well.

Experiment 2: Surface marker screening of a cancer cell line

If you don't yet know which proteins are typically expressed by a subpopulation of interest, BD Lyoplate™ cell surface marker screening panels provide a comprehensive and efficient solution for profiling cancer cells for hundreds of human or mouse cell surface markers by flow cytometry. Deciphering the cell surface proteome enables researchers to define strategies for the analysis and isolation of targeted cells from heterogeneous populations for functional studies, drug screening, and in vivo animal studies.[3,4]

Both the human (Cat. No. 560747) and mouse (Cat. No. 562208) screening panels contain three plates. Each well contains lyophilized, purified antibody to one cell surface marker or isotype control. The process is illustrated in Figure 2.

Figure 3 shows the results when MCF-7 breast cancer cells were analyzed for surface marker expression using the BD Lyoplate™ Human Cell Surface Marker Screening Panel (Cat. No. 560747). The heatmap summarizes the expression of selected markers, from those expressed almost universally to those expressed rarely or never. The plots show different patterns of expression for selected markers.

Easy to use, simple to maintain, and affordable, the BD Accuri C6 personal flow cytometer is equipped with a blue laser, a red laser, two light scatter detectors, and four fluorescence detectors. A compact design, fixed alignment, and pre-optimized detector settings result in a system that is simple to use. A nonpressurized fluidics system enables kinetic measurements in real time. For walkaway convenience, the optional BD CSampler™ accessory (Cat. No. 653124) offers automated sampling from 24-tube racks or multiwell plates.

Visit **bdbiosciences.com** for more information.

Immunophenotyping Cancer Cells Using Flow Cytometry

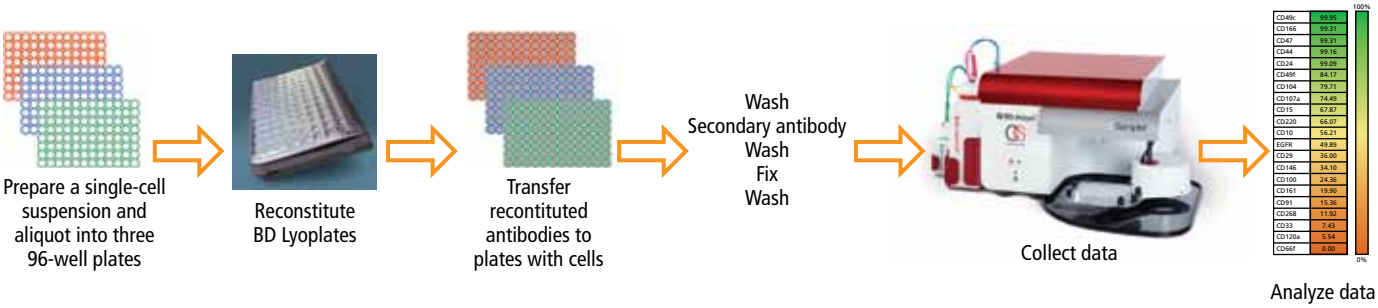
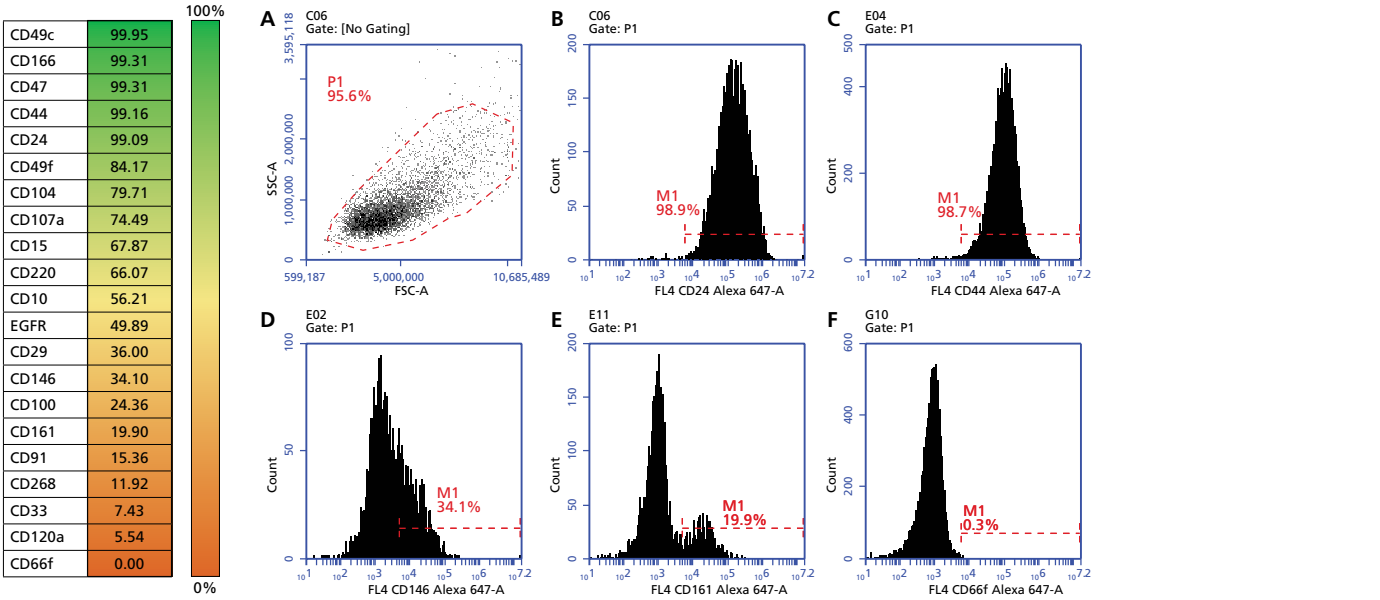


Figure 2. BD Lyoplate surface marker screening workflow



CD49c	99.95
CD166	99.31
CD47	99.31
CD44	99.16
CD24	99.09
CD49f	84.17
CD104	79.71
CD107a	74.49
CD15	67.87
CD220	66.07
CD10	56.21
EGFR	49.89
CD29	36.00
CD146	34.10
CD100	24.36
CD161	19.90
CD91	15.36
CD268	11.92
CD33	7.43
CD120a	5.54
CD66f	0.00

Figure 3. Surface marker screening of breast cancer cells

A single-cell suspension of MCF-7 cells (human breast adenocarcinoma; ATCC) was prepared using BD Accutase Cell Detachment Solution (Cat. No. 561527). Fifty million cells were aliquoted in three 96-well plates (~180K cells/well) and stained with the BD Lyoplate Human Cell Surface Marker Screening Panel (Cat. No. 560747). After staining, cells were fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655). Plates were sealed and stored at 4°C. Cells were acquired within 3 days using the BD CSampler accessory (Fast speed, 1 wash cycle, 2 agitation cycles every 4 wells), which processed each plate in 2.5–3 hours. Cells were analyzed using BD Accuri C6 software. **Results: (A)** Cells were initially gated based on light scatter properties. **(B-F)** M1 gates were drawn based on isotype controls. **(B, C)** Almost all cells expressed both CD24 and CD44. **(D)** Cells expressed varying levels of CD146. **(E)** CD161 showed a bimodal distribution. **(F)** Almost no cells expressed CD66f. A heatmap (left) summarizes the expression of selected markers tested.

Ordering Information

Description	Cat.No.
BD Accuri™ C6 Flow Cytometer System	**653118**
BD CSampler™ Automated Sampling System	**653124**
BD Pharmingen™ Mouse Anti-Human CD24 PE	**555428**
BD Pharmingen™ Mouse Anti-Human CD44 APC	**559942**
BD Lyoplate™ Human Cell Surface Marker Screening Panel	**560747**
BD Lyoplate™ Mouse Cell Surface Marker Screening Panel	**562208**
BD™ Accutase™ Cell Detachment Solution	**561527**
BD Cytofix™ Fixation Buffer	**554655**

References

1. Murohashi M, Hinohara K, Kuroda M, et al. Gene set enrichment analysis provides insight into novel signaling pathways in breast cancer stem cells. *Br J Cancer.* 2010;102:206-212.
2. Sheridan C, Kishimoto H, Fuchs RK, et al. $CD44^+CD24^-$ breast cancer cells exhibit enhanced invasive properties: An early step necessary for metastasis. *Breast Cancer Res.* 2006;8:R59.
3. Sukhdeo K, Paramban RI, Vidal JG, et al. Multiplex flow cytometry barcoding and antibody arrays identify surface antigen profiles of primary and metastatic colon cancer cell lines. *PLoS One*. 2013;8:e53015. doi: 10.1371/journal.pone.0053015
4. Lathia JD, Li M, Sinyuk M, et al. High-throughput flow cytometry screening reveals a role for junctional adhesion molecule a as a cancer stem cell maintenance factor. *Cell Rep*. 2014;6:117-29.

23-16934-00

BD Biosciences
bdbiosciences.com