# Analysis of Cytokine Expression in Cancer Cell Lines Using Flow Cytometry

## Cancer Biology Applications on the BD Accuri™ C6

### Features

- Quantify cytokine production of a cell population using BD CBA
- Simplify setup and analysis using BD CBA kits and free software templates
- Analyze cytokine expression at the single-cell level using intracellular flow cytometry

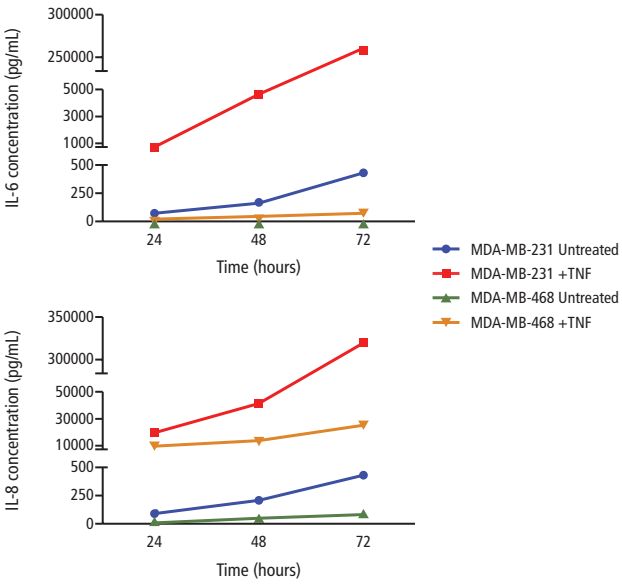



**Figure 1.** Cytokine expression of breast cancer cell lines using BD CBA

MDA-MB-231 and MDA-MB-468 cells (human epithelial breast adenocarcinoma; ATCC) were treated with 10 ng/mL of BD Pharmingen™ TNF and cell culture supernatants were collected at 24 hour intervals. 50 µL of sample were tested with the BD CBA Human Inflammatory Cytokine Kit according to the kit instructions and acquired on a BD Accuri C6 using the free, downloadable BD CBA Kit template. **Results:** Exported data was analyzed using FCAP Array™ software v3.0 to determine cytokine concentrations by interpolation from standard curves, and time course graphs were plotted. MDA-MB-231 cells expressed IL-6 and IL-8 at basal levels (blue lines). Upon treatment with TNF, the concentration of IL-6 and IL-8 dramatically increased over 72 hours (red lines). MDA-MB-468 cells expressed lower basal levels of IL-6 and IL-8 (green lines), and upregulated IL-8 and modestly upregulated IL-6 expression upon TNF treatment (orange lines).

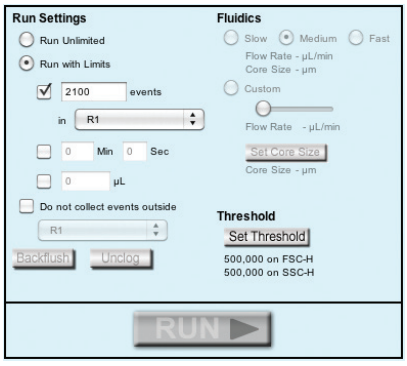



**Figure 2.** Run settings from the BD CBA Kit template

The preconfigured run settings and gates in the free, downloadable BD CBA Kit template facilitate quick and easy analysis of BD CBA bead clusters.

The invasiveness, metastasis, and growth of cancer cells are tied to pro-inflammatory cytokines such as IL-6 and IL-8. Using traditional methods such as ELISA, PCR, or Western blot to study cytokine expression can consume substantial time, labor, budget, and sample. Flow cytometry is especially well suited for cytokine studies because it can rapidly quantify multiple cytokines, either for a population as a whole or at the single-cell level.

Bead-based flow cytometric immunoassays are powerful methods of quantifying cytokines because they allow researchers to multiplex many analytes with very little sample. A BD™ Cytometric Bead Array (CBA) assay contains a cocktail of beads that are mixed with samples along with detection antibodies using flow cytometry. In essence, BD CBA on a BD Accuri™ C6 flow cytometer is like running ELISAs for up to 30 cytokines at once.

Figure 1 shows cytokine analyses performed on the supernatant of two breast cancer cell line cultures using the BD CBA Human Inflammatory Cytokine Kit on the BD Accuri C6. MDA-MB-231 cancer cells showed a stronger IL-6 and IL-8 cytokine response to TNF stimulation compared to MDA-MB-468 cells.

This example shows how the BD Accuri C6 and BD CBA provide a streamlined solution for cancer cell cytokine analysis. Both IL-6 and IL-8 (along with four other cytokines) were analyzed simultaneously from a single sample. A free, downloadable BD Accuri™ C6 software template was used to supply preconfigured settings and gates (Figure 2). Finally, the BD CBA kit provided a preconfigured panel of analytes for ultimate ease of use. The workflow is not only simple but also minimizes errors (Figure 3).

BD CBA, like ELISA, measures secreted proteins in the supernatant of cell populations. Tumor samples and cancer cell lines can be heterogeneous, raising the question whether all the stimulated cells are producing small amounts of cytokine, or specific subsets of cells are secreting large amounts. Intracellular flow cytometry can answer this question by simultaneously analyzing multiple cytokines within phenotypic populations at the single-cell level.

Figure 4 shows cytokine analyses of the same two breast cancer cell line cultures using intracellular flow cytometry on the BD Accuri C6. Consistent with the CBA data, TNF treatment induced more MDA-MB-231 cells than MDA-MB-468 cells to express both IL-6 and IL-8. Surface marker analysis also verified that most MDA-MB-231 cells expressed the $CD44^{+}CD24^{-}$ cancer stem cell phenotype, while MDA-MB-468 cells expressed both CD44 and CD24, confirming a correlation between a more aggressive immunophenotype and pro-inflammatory cytokine expression. Finally, the experiment revealed that only 20%–35% of the cells actually produced each cytokine.

BD CBA and intracellular flow are complementary techniques that can be used for a comprehensive analysis of cytokine expression in heterogeneous samples such as cancer cells.

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

# Analysis of Cytokine Expression in Cancer Cell Lines Using Flow Cytometry

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.

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 offers automated sampling from 24-tube racks or multiwell plates.

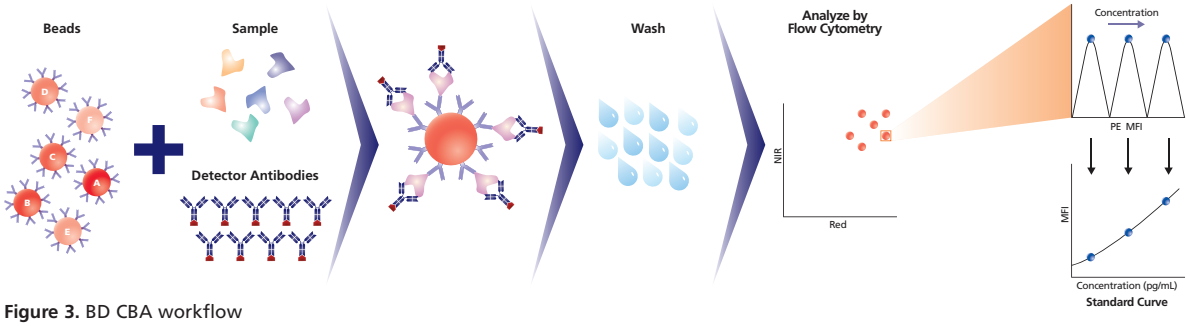
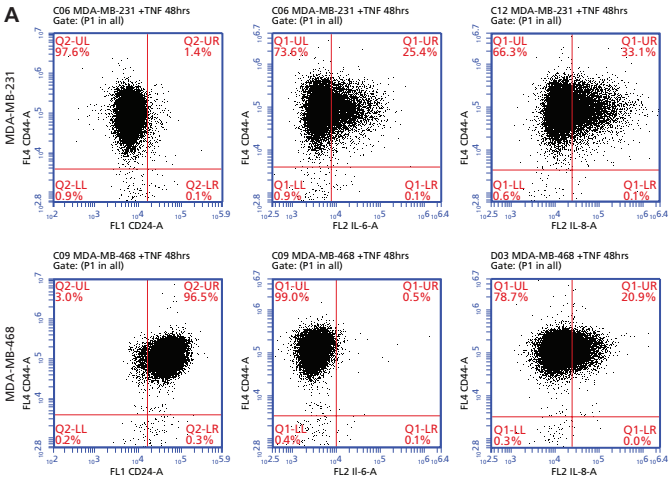



**Figure 3.** BD CBA workflow

With BD kits and free downloadable software templates, BD CBA and the BD Accuri C6 provide a streamlined solution for cancer cell cytokine analysis.



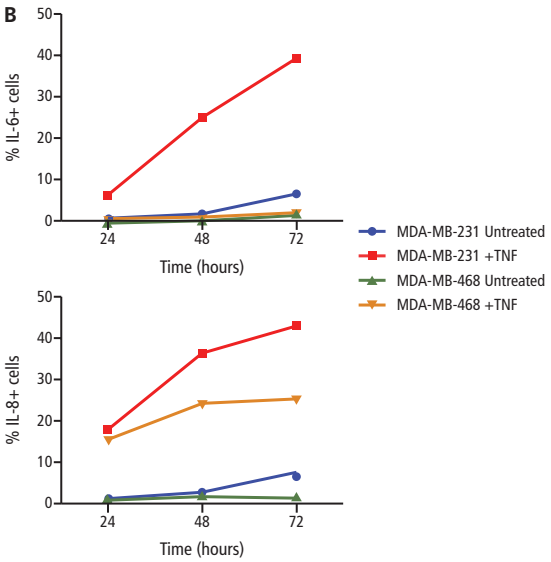




**Figure 4.** Cytokine expression of breast cancer cell lines using surface and intracellular flow cytometry

MDA-MB-231 and MDA-MB-468 cells were treated with TNF. At 24-hour intervals, cells were treated with BD GolgiStop™ protein transport inhibitor, dissociated using BD Accutase® Cell Detachment Solution, and stained with CD24 FITC and CD44 APC. Cells were then fixed and permeabilized using the BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution Kit before staining with PE-conjugated antibodies to IL-6 or IL-8. Isotype and fluorescence-minus-one controls were used for each cell line and treatment to draw the gates accurately. **Results: A.** MDA-MB-231 (upper plots) showed a cancer stem cell phenotype ($CD44^+CD24^-$) whereas MDA-MB-468 (lower plots) expressed both CD44 and CD24. Upon treatment with TNF, a discrete subset of MDA-MB-231 cells expressed IL-6 and IL-8. A distinct subset of MDA-MB-468 cells expressed IL-8 but, consistent with the BD CBA data, very few cells expressed IL-6. **B.** Time course analyses of IL-6 and IL-8 expression using intracellular flow cytometry showed a similar trend to that observed using BD CBA.

## Ordering Information

All kits and their associated software templates are available at bdbiosciences.com/go/templates.

| Description | Cat.No. |
|---|---|
| **BD Accuri™ C6 Flow Cytometer System** | **653118** |
| **BD CSampler™ Automated Sampling System (optional)** | **653124** |
| **BD™ CBA Human Inflammatory Cytokine Kit** | **551811** |
| **BD Pharmingen™ TNF, Recombinant Human** | **554618** |
| **BD™ CBA FCAP Array Software v3.0** | **652099** |
| **BD GolgiStop™ Protein Transport Inhibitor** | **554724** |
| **BD Accutase® Cell Detachment Solution** | **561527** |
| **BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution Kit** | **554714** |
| **BD Pharmingen™ Mouse Anti-Human CD24 FITC** | **555427** |
| **BD Pharmingen™ Mouse Anti-Human CD44 APC** | **559942** |
| **BD Pharmingen™ Rat Anti-Human IL-6 PE** | **559331** |
| **BD Pharmingen™ Mouse Anti-Human IL-8 PE** | **554720** |

Class 1 Laser Product.

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