

Assessing NK cell cytotoxicity against tumor cells using personal flow cytometry

Immuno-oncology applications for the BD Accuri™ C6 Plus flow cytometer

Features

- Quantitatively assess targeted tumor cell death at the single-cell level
- Simultaneously assess degranulation of NK cells
- Discriminate tumor cells from NK cells in co-culture
- Obtain accurate cell counts and concentrations without counting beads

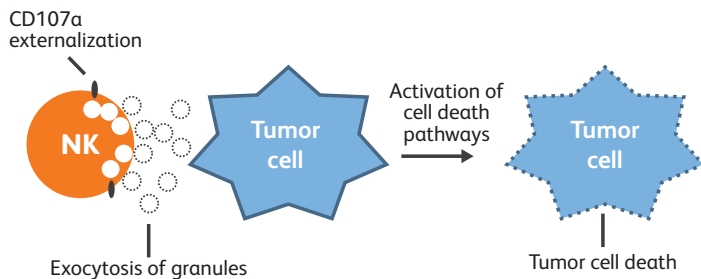


Figure 1. Model assay to evaluate NK cell activation and cytotoxicity against tumor cells

NK cells are activated and then co-cultured with tumor cells. Degranulation of NK cells is measured by expression of CD107a on the cell surface. Cytotoxicity against tumor cells is measured by analysis of cell death using BD Pharmingen™ APC Annexin V and BD Pharmingen™ 7-AAD staining.

When cytotoxic natural killer (NK) cells are attacking tumor cells, it is important to measure both activation of the NK cells and toxicity against the tumor cells. Conventionally, radioactive (chromium) and colorimetric (MTT or calcein) assays have been used to measure NK-mediated target cell death. However, these bulk assays measure only a single parameter at a time, thus providing limited insights about the activation of effector cells and the mechanisms involved in the cytotoxic process.

Multicolor flow cytometry can supplement these assays by offering a more comprehensive understanding of these mechanisms, and by further illuminating NK cell function (such as cell degranulation or cytokine production) while accurately discriminating the NK cells from the target cell population. On the BD Accuri™ C6 Plus personal flow cytometer, with two lasers and four fluorescence parameters, you can perform these studies right on your benchtop. In addition, because the fluidics system accurately monitors the sample volume acquired per run, you can calculate absolute counts or sample concentrations per μL without the use of counting beads. These absolute counts are more precise and far less tedious than manual counts, and can differentiate between the two cell populations.



The experimental model is shown in Figure 1. When NK cells are activated—by a sensitive target tumor cell line, for example—they release their lytic granule contents (granzymes and perforin) onto the surface of the target cell. The released contents cause irreversible cell damage and lead to cell death through apoptosis, which can be measured using Annexin V and 7-amino actinomycin D (7-AAD). Degranulation of NK cells can be recognized by CD107a externalization. You can assess these parameters in a single assay using three of the four fluorescence channels of the BD Accuri C6 Plus system.

The broad dynamic range of the BD Accuri C6 Plus allows resolution of cell types of different sizes using forward and side scatter. By labeling one of the co-cultured cell populations with a fluorescent marker, you can use the fourth fluorescence channel to further differentiate the NK cells from the target cell population. In Figure 2A, K562 chronic myelogenous leukemia cells have been labeled with PKH67, a green fluorescent lipophilic dye. Plotting PKH67 vs side scatter (SSC) results in a clear separation between the K562 and NK cells, which can be used to gate the cells for further analysis.

The remaining panels of Figure 2 contain the cytotoxicity results for K562 tumor cells (identified as PKH67⁺). Figure 2B shows that compared to tumor cells cultured alone (left plot), tumor cells co-cultured for four hours with activated NK cells progressed from live (lower left quadrant) to early apoptotic (lower right) and then to late apoptotic (upper right). A higher ratio of NK to K562 cells (4:1 vs 1:1, right and middle plots) was associated with higher cytotoxic activity (8.9% vs 18.7% live K562 cells).

The graph in Figure 2C reiterates these results, and further shows that the number of viable cells decreased over time in both conditions. The volumetric counting ability of the BD Accuri C6 Plus flow cytometer allowed direct quantification of the number of viable tumor cells.

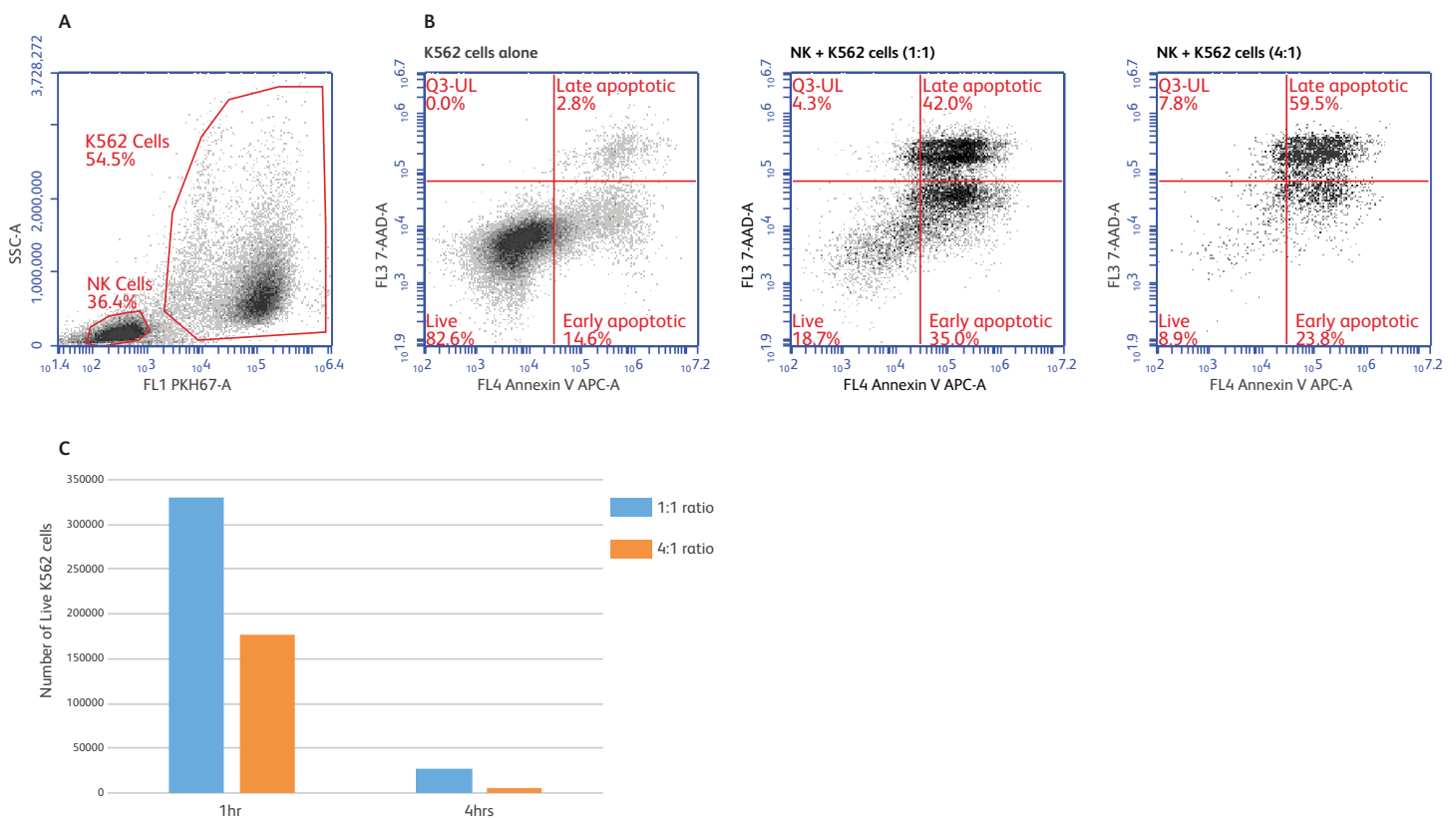


Figure 2. Cytotoxicity of activated NK cells against K562 tumor cells

NK cells from a normal donor were enriched with the BD IMag™ Human NK Cell Enrichment Set (Cat. No. 557987). Enriched NK cells were activated with NK Cell Activation/Expansion Kit, human (Miltenyi Biotec) and expanded in complete media containing BD Pharmingen™ Recombinant Human IL-2 (Cat. No. 554603) for one week. K562 cells (human chronic myelogenous leukemia, ATCC CCL-243) were labeled with PKH67 Green Fluorescent Cell Linker (Millipore Sigma). Activated NK cells were then added to PKH67-labeled K562 cells at a ratio of either 4:1 or 1:1 and acquired after one or four hours. **Results:** **A.** Side scatter combined with PKH67 fluorescent signal provides a clear separation of NK cells from PKH67⁺ K562 cells. **B.** After gating on PKH67⁺ K562 cells, cell viability was assessed by analyzing BD Pharmingen™ APC Annexin V and BD Pharmingen™ 7-AAD (Cat. Nos. 550475 and 559925). After four hours of culture, percentages of early apoptotic (Annexin V⁺7-AAD⁻) and late apoptotic (Annexin V⁺7-AAD⁺) cells increased, compared to K562 cells cultured alone. A higher effector-to-target cell ratio (4:1) resulted in more cytotoxicity. **C.** The absolute number of live (Annexin V⁻7-AAD⁻) K562 cells, counted volumetrically, with time and effector cell ratio.

Figure 3 shows the assessment of the activation of NK cells (identified as PKH67⁻), expanded with IL-2 and analyzed from the same tube as Figure 2. Few NK cells, cultured alone, expressed CD107a on the cell surface (4.4%, second plot). Percentages of CD107a⁺ cells in the co-cultures, however, increased to 13.3% at 1 hour (third plot) and 39.4% at 4 hours (fourth plot), suggesting that CD107a was externalized upon stimulation with K562 cells.

The use of flow cytometry to assess cytotoxicity allows you to probe deeply into immuno-oncological processes. With two light scatter and four fluorescence parameters, the BD Accuri C6 Plus flow cytometer can provide more information than conventional bulk assays by assessing immune cell activation and tumor cell death simultaneously. To learn how you could expand this assay if you had a third laser and additional fluorescence channels, refer to our companion product information sheet for the BD FACSCelesta™ flow cytometer.

Easy to use, simple to maintain and affordable, the BD Accuri C6 Plus personal flow cytometer is equipped with a blue laser, a red laser, two light scatter detectors and four fluorescence detectors. A compact and transportable design, fixed laser alignment, preoptimized detector settings and automated instrument QC result in a system that is straightforward to operate. For walkaway convenience, the optional BD CSampler™ Plus accessory offers automated sampling from 24-tube racks or multiwell plates.

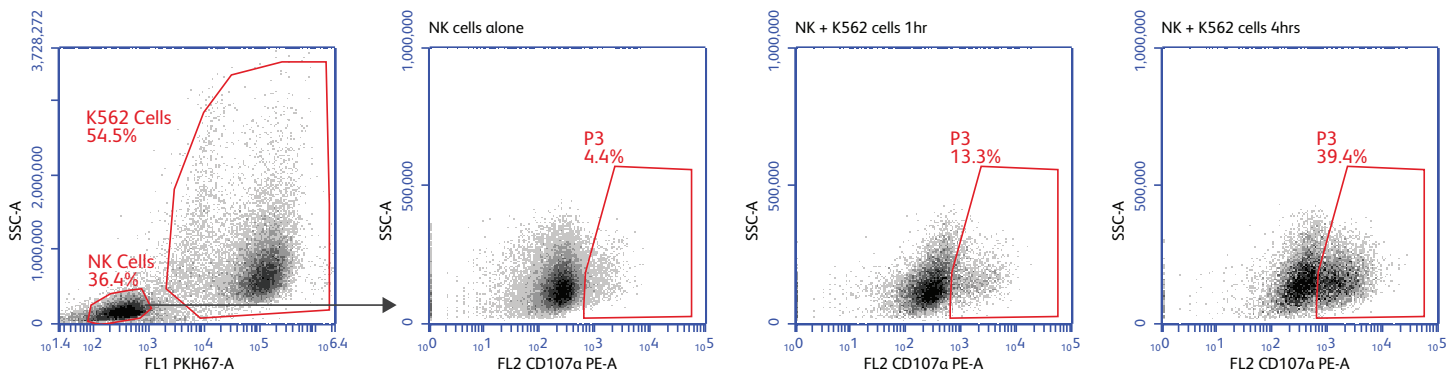


Figure 3. Degranulation of activated NK cells following co-culture with K562 tumor cells

NK cells were purified, expanded and activated from blood, and cultured alone or with K562 cells, and acquired from the same tube as described in Figure 2. BD Pharmingen™ PE Mouse Anti-Human CD107a (Cat. No. 555801) was added to the culture media, as described in the literature. After gating on PKH67⁻ NK cells, expression of CD107a was monitored after one and four hours of co-culture with K562 cells at a 1:1 ratio. The percentage of CD107a⁺ NK cells increased (third and fourth plots) over time, compared to NK cells cultured alone (second plot).

Ordering information: Systems and software

Description	Cat. No.
BD Accuri™ C6 Plus Flow Cytometer System	660517
BD Accuri™ C6 Plus Workstation Computer and Software	661391
BD CSampler™ Plus Automated Sampling System (optional)	660519

Ordering information: Reagents

Description	Cat. No.
BD IMag™ Human NK Cell Enrichment Set	557987
BD Pharmingen™ Recombinant Human IL-2	554603
BD Pharmingen™ APC Annexin V	550475
BD Pharmingen™ 7-AAD	559925
BD Pharmingen™ PE Mouse Anti-Human CD107a	555801

Class 1 Laser Product.

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