# Efficient Europium Labeling of Hodgkin-Derived Cell Line L540cy Using the Gene Pulser® II System with RF Module for Use in a Lymphokine-Activated Killer Cell Cytolytic Assay

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Cytokines have been described as soluble mediators with defined functions in the development and regulation of the immune system. Interleukin-2 (IL-2) is a cytokine produced by activated T-lymphocytes that shows stimulating activity on a large subset of leucocytes and especially induces clonal proliferation of T cells and natural killer (NK) cells (Rosenberg *et al.*1984). The cytolytic activity of mature NK cells can further be increased *in vitro* and *in vivo* by exposure to IL-2, IL-12 or interferon α/β; these so-called lymphokine-activated killer (LAK) cells exhibit powerful cytotoxic activity against isolated primary tumor cells or disseminated growing tumors in mouse models (Rosenberg *et al.* 1985).

To determine the immunological effector functions of LAK cells against cellular targets, different cytotoxicity experiments have been established in recent years. As $^{51}Cr$ tests can only be performed in sequential experiments, the use of lanthanides (europium, samarium, terbium) in time-resolved fluorometry has been shown to solve this problem (Blomberg *et al.* 1986). Using this established method, the release of europium ($Eu^{3+}$) from labeled cells can easily be determined. For this purpose, we electropulsed different tumor cells with Eu-DTPA chelate complexes. In this *in vitro* test system, our aim was to document the activity of activated NK cells against their labeled target cells: europium will be released after cellular lysis and can be measured in the medium. After addition of an enhancement solution, the emitted, time-resolved fluorescence can then be evaluated in a fluorometer at 613 nm to determine the specific cytotoxicity of the LAK cells (Blomberg *et al.* 1986).

We measured the cytolytic activity of cytokine-activated natural killer cells against the Hodgkin-derived cell line L540cy (Diehl *et al.* 1982). Electroporation was performed using the Gene Pulser II system combined with the radio frequency (RF) module (Bio-Rad).

## Method

- Cultivate the tumor cells (RPMI 1640 medium, 10% fetal bovine serum, 50 U penicillin/streptomycin)
- Pellet cells by centrifugation (250 x g, 10 min, RT), wash cells and calibrate them to a density of 1 x $10^7$/ml
- Repeat centrifugation step and add 1 ml labeling solution (2 mM EuCl/10 mM DTPA)
- Transfer 800 µl into a Gene Pulser cuvette (0.4 cm gap between the electrodes)
- Program Gene Pulser II and RF module as follows: 200 V, 2 ms pulse duration, 100% modulation, 40 kHz, 5 pulses, 1 s pulse interval
- Remove cells from cuvette after electroporation and incubate for 10 min at 37 °C
- Wash cells 5x with culture medium
- Calibrate Ficoll-gradient separated LAK cells and enrich them by magnetic cell separation (MACS) to a density of $10^6$/ml
- Add tumor cells in a ratio 1:40 and incubate 2 hr at 37 °C in 5% $CO_2$
- Centrifugate (250x g, 10 min, RT), add 200 ml Delfia™ enhancement solution (LKB-Wallac, Turku, Finland) to 20 ml supernatant
- Perform fluorometric determination of europium release at 613 nm
- Cytotoxicity is calculated as:

$$\frac{x - SR}{MR - SR} \times 100 = \% \text{ specific lysis}$$

where x = sample counts
SR = spontaneous release counts
MR = maximum release counts

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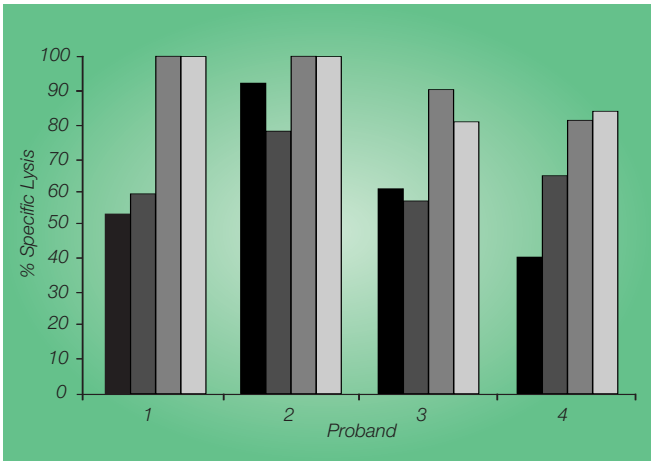


*Fluorometric determination of released europium after specific lysis of L540cy cells using LAK cells of different probands. Lysis of L540cy cells before MACS of LAK cells stimulated by IL-2 ( ■ ) or by IL-2 and IL-12 ( ■ ) and after MACS of LAK cells stimulated by IL-2 ( ■ ) or by IL-2 and IL-12 ( ■ ).*

**FACS Analysis of MACS-Sorted and Unsorted Peripheral Blood Lymphocytes**

| PROBAND | BEFORE MACS (%) | AFTER MACS (%) |
|---|---|---|
| 1 | 10 | 65 |
| 2 | 26 | 61 |
| 3 | 18 | 77 |
| 4 | 8 | 47 |

## Results

Having isolated peripheral blood lymphocytes of different probands over a Ficoll gradient, the whole population of either peripheral blood lymphocytes (before MACS) or CD56-sorted peripheral blood lymphocytes (after MACS) was stimulated with 650 U IL-2 (±100 U IL-12) for 48 hr. Thus-generated LAK cells were used in parallel to assess europium release (see figure).

MACS sorting of NK cells results in a significantly higher LAK cell activity of all probands evaluated against the Hodgkin-derived cell line L540cy. The percentage of NK cells isolated from the peripheral blood of the probands before and after magnetic cell separation has been determined by fluorescence-activated cell sorting (FACS) analysis (see table).

## References

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