

## 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  $\alpha/\beta$ ; 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  $^{51}\text{Cr}$  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 ( $\text{Eu}^{3+}$ ) 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).

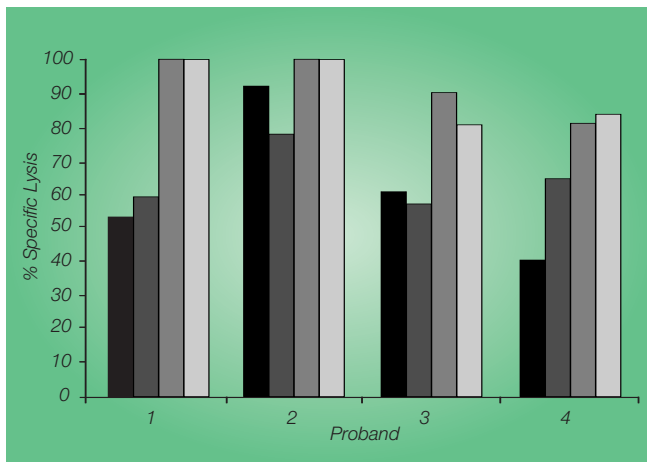
We measured the cytolytic activity of cytokine-activated natural killer cells against the Hodgkin-derived cell line L540cy (Diehl *et al.* 1982). Electroporation was performed using the Gene Pulser II system combined with the radio frequency (RF) module (Bio-Rad).

### 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  $1 \times 10^7/\text{ml}$
- Repeat centrifugation step and add 1 ml labeling solution (2 mM  $\text{EuCl}_3/10 \text{ mM DTPA}$ )
- Transfer 800  $\mu\text{l}$  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  $10^6/\text{ml}$
- Add tumor cells in a ratio 1:40 and incubate 2 hr at 37 °C in 5%  $\text{CO}_2$
- 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:

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

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



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 ( $\pm 100$  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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