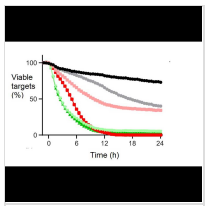


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# Time-lapse killing assay (monolayer - IncuCyte)

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ABSTRACT

Fluorescent target cells are plated in a monolayer in a 96 well plate. Effector cells are added to that plate and time-lapse imaging in combination with fluorescent indicators of cell death reveal the dynamics of target cell death.

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BEFORE STARTING

If using adherent target cells, label them and plate them the day before the assay.

If using non-adherent target cells, the day of the assay, start by pre-coating a plate with poly-L-ornithine.

1 Target cells are fluorescently labelled to differentiate them from effector cells.

1.1 *Option 1:* Target cells are labeled using an amine-reactive dye (CellTrace Far Red Proliferation Kit, Cat. No:C34564, Thermo Fisher) according to the manufacturer's instructions.

1.2 *Option 2:* Target cells are stably transduced with a red fluorescent protein (IncuCyte NucLight Red Lentiviral Reagent - EF1α Puro, Cat. No. 4625, Essen Bioscience) according to the manufacturer's instructions.

1.3 *Option 3:* Target cells are stably transfected with a green fluorescent protein (GFP). In this situation, a red cell death indicator should be used (IncuCyte Caspase 3/7 Red Apoptosis Assay Reagent, Cat. No. 4704, Essen BioScience).

2 Labelled target cells are plated in a monolayer in a 96 well flat-bottom plate (Cat. No: 353072, Corning). Adherent targets are allowed to adhere overnight. Non-adherent target cells can be immobilised on plates pre-coated with 0.01% poly-L-ornithine (pre-coated for 2 h at room temperature; Cat. No. A-004-C, Fisher Scientific). Cell numbers are titrated for each target, but are generally set at $2 \times 10^4$ cells/well in 100 µL.

3 An indicator of cell death is added to the media (1/1000 dilution; IncuCyte Caspase-3/7 Green Apoptosis Assay reagent, Cat. No:4440, Essen BioScience).

4 Enriched NK cells are added to the target cell monolayer at a 2:1 effector:target ratio, bringing the final volume to 200 µL.

5 Plates are placed in an incubator equipped with a time-lapse microscope with 4x, 10x and 20x lenses (IncuCyte Zoom or S3, Sartorius Inc.) for 48 hours. Images are recorded at 30 min intervals.

6 Image analysis is performed in the Incucyte software. Live targets (Target cell fluorescence+ Caspase3/7-) are detected at each time point. The percentage of live targets at any given time point is normalized for the number of live targets in each well at the starting time point (t = 0 h), and further normalized to the growth of targets growing alone. This data is then plotted in GraphPad Prism.



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