

# Proliferation Assay

Erika Wissinger, Imperial College London, UK

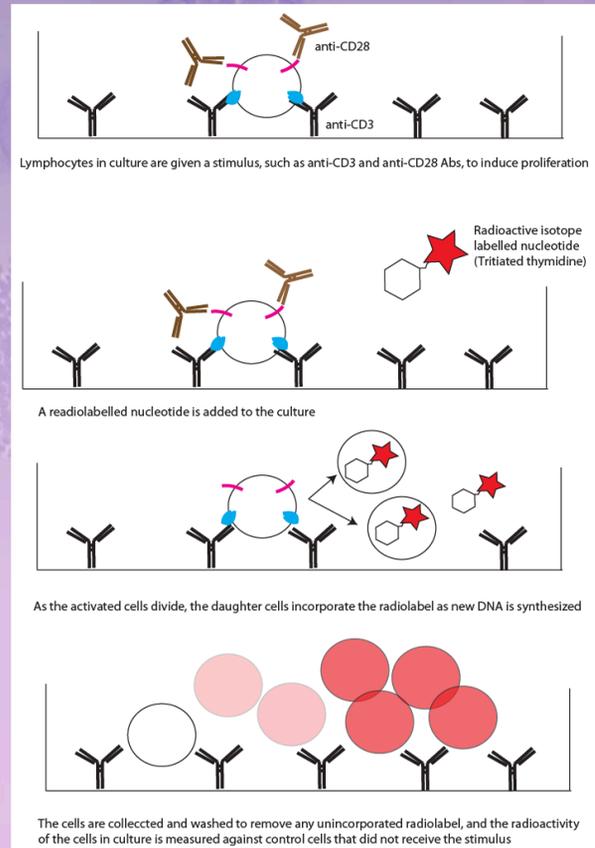


## Objectives of the assay

The *in vitro* proliferation assay can be used to determine whether or not cells are triggered to divide after exposure to a specific stimulus, or to assess differences between cell populations in their ability to divide in response to the same stimulus.

## Procedure

- Cells in culture are given a specific stimulus. For **T cells**, this is commonly plate-bound **anti-CD3 antibody** in combination with soluble **anti-CD28 antibody** (see figure). Other commonly used compounds to stimulate T-cell proliferation are **Con A**, **PHA**, and **PMA** and **ionomycin**.
- A radio-labelled nucleotide is then added to the culture media. The most commonly used is 3H-thymidine. As the cells are stimulated to divide, they utilise the radio-labelled nucleotide in the culture media and incorporate it into their newly-synthesized DNA. Each successive generation of daughter cells will incorporate more of the radio-labelled nucleotide into its DNA.



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3) After this incubation (generally 24-48 hours), the cells are removed from the culture media by centrifugation and washed to remove any free radio-labelled nucleotide that has not been incorporated into the cells' DNA. The total radioactivity is measured, and compared against a control group of cells that did not receive the proliferation-inducing stimulus.

## Limitations and alternative approaches

The proliferation assay as described above provides information about the proliferation of a population of cells as a whole, rather than about individual cells. This assay is relatively quick and inexpensive to perform, but does involve the use of radioisotopes, and a specialized machine to read the level of radioactivity present in the samples.

An alternative experimental approach which provides more information about individual cells is to label cells in culture with a fluorescent marker, such as carboxyfluorescein succinamidyl ester (**CFSE**), which is diluted in each generation of daughter cells as they divide, and can be measured by **flow cytometry**.