**[³H]-Thymidine Proliferation Assay**

**Principle**

[³H]-Thymidine is a labeled DNA precursor. Before a cell divides, its DNA is replicated and precursors are incorporated, thus if the cells are proliferating and [³H]-Thymidine is added to the culture, it will be incorporated into the cells’ DNA. The amount of [³H]-Thymidine incorporated into the DNA is measured with a scintillation counter. The level of the radioactive signal depends on the proliferation rate – the higher the proliferation rate, the more cells can be harvested thus the more radioactive DNA and the higher signal. Usually, an inhibitor of proliferation is added to the culture. Inhibition of proliferation in the presence of such an agent is calculated from the following formula:

\[
\text{Inhibition of Proliferation [%]} = \frac{\text{cpm (untreated)} - \text{cpm (treated)}}{\text{cpm (untreated)}}
\]

**Objective**

- to measure an effect of an agent on cell proliferation
- to check an effect of cytotoxic drugs on tumor cells
- to determine the cycle and its phases
- to measure the rate of DNA synthesis
- to detect antigen-specific T-cell proliferation in culture

**Procedure**

- 3H-Thymidine is added to the cells in culture

- growth inhibitor is added to one of the cell cultures, others are incubated without any additional agents

- cells are incubated for a certain period of time (e.g. 24h)

- cells are harvested with bidest water: the cells and organelles burst and the cell’s DNA is set free

- the cell fragments and DNA are passed through a filter membrane, only particles of a certain diameter can pass the filter. Thus, intact DNA will be too big to pass the filter and will stay on the filter membrane

- radioactivity of the membrane is measured

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Aptosis, Cell Death and Cell Proliferation by Roche Applied Science, 3rd edition
Example

Taken from:

The purpose of the experiment was to investigate the influence of NOX5-derived ROS on cell proliferation and apoptosis in esophageal adenocarcinoma cells. Cells were cultured in different conditions, which included: pulsed acid treatment (1 hour at pH 4.0 followed by 24 hours at pH 7.2), knockdown of NOX5, different concentrations of H₂O₂, and combined conditions. Cell proliferation was assessed with ³H-TdR uptake assay.

Conclusions from the experiment:

(A)
-Knockdown of NOX5-S significantly decreased basal ³H-thymidine incorporation
-Pulsed acid treatment significantly increased ³H-thymidine incorporation, and the increase was blocked by knockdown of NOX5-S

(B)
-Low doses of H₂O₂ significantly increased ³H-thymidine incorporation
-High doses of H₂O₂ significantly decreased ³H-thymidine incorporation (low doses of H₂O₂ stimulate cell proliferation, whereas high doses of H₂O₂ inhibit cell proliferation)

(C)
-The decrease in cell proliferation induced by knockdown of NOX5 was restored by 10-13M H₂O₂

References
- http://www.celldeath.de/apometh/prolif.html
- Apoptosis, Cell Death and Cell Proliferation by Roche Applied Science, 3rd edition