

Thymidine incorporation assay (48-well cluster plate)

Solutions:

³H-Thymidine: 1mCi/ml, 1:40 diluted in PBS, [0.025 mCi/ml]

Methanol

5% TCA

0.3 M NaOH

Rotiszint ECO Plus (Roth Art. 0016)

Growth and basal medium depending on the cell type

Note: This protocol is optimized for human vascular endothelial cells!

Protocol (for HUVEC):

- plate cells with a density at $5-7 \times 10^3$ cells/well in growth medium (EGM)
- incubate cells over night [if urgent, plate cells in the morning, change growth medium against basal medium (EBM) in the early afternoon]
- change EGF against EBM (for HUVEC: EBM +1-2% FCS)
- incubate 24h
- change medium again and add factors (growth factors, inhibitors, etc)
- incubate for 18h
- add 10 μ l ³H-Thymidine solution [0.025mCi/ml] per well (=0.25 μ Ci)
- incubate another 6 h at 37°C
- Washing steps: (250 μ l/well)

PBS	1x
MeOH	2x 5min
TCA	2x 10min
H ₂ O	1x
- lyse cells in 250 μ l 0.3M NaOH per well
- transfer 2.5 ml ECO Plus into the appropriate scintillation vials
- transfer cell lysats into the scintillation vials
- count by liquid scintillation (β -counter; Beckmann Instruments)