

## **Thymidine incorporation assay (48-well cluster plate)**

### **Solutions :**

$^3\text{H}$ -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  $10^3$   $^3\text{H}$ -Thymidine solution [0.025mCi/ml] per well (=0.25 $\mu$  Ci)
- incubate another 6h at 37°C
- Washing steps: (250 $\mu$ l/well)

PBS	1x
MeOH	2x 5min
TCA	2x 10min
H <sub>2</sub> O	1x
- lyse cells in 250 $\mu$ l 0.3M NaOH per well
- transfer 25 ml ECO Plus in to the appropriate scintillation vials
- transfer cell lysats into the scintillation vials
- count by liquid scintillation ( $\beta$ -counter; Beckmann Instruments)