Thymidine incorporation assay with Balb/c 3T3 cells (96-well cluster plate)

Material:

- 1% Gelatine in ddH_2O, PBS
- Growth medium: DMEM (4,5g Glucose), 1% Pen/Strep, 1% Glutamin, 10% Calf Serum (HyClone: Cat.No. SH30072.03)
- Basal medium: DMEM, 1%Pen/Strep, 1% Glutamin, 2,5% CS
- ^{3}H-Thymidine 1mCi/ml, 1:40 verdünnt in PBS (0,025mCi/ml)
- PBS, Methanol, 5% TCA, ddH_20, 0,3M NaOH (use at 4°C!)

Protocol:

- Expand Balb/c 3T3 cells in growth medium (10% CS) and use cells at a confluence of about 70-90%
- coat a 96-well cluster plate with gelatine (1% in water) and incubate for 15 min at 37°C

✍ fill at least the outer row with PBS because of evaporation over the long time

- plate cells with a density at 2 x 10^3 cells/well in basal medium (2.5% CS)
- incubate cells 7 to 14 d at 37°C and 5% CO_2
- stimulate the cells with the desired growth factors (as positive control 10µl CS is used)
- add 10?l ^{3}H-Thymidine solution [0.025mCi/ml] per well (=0.25?Ci)
- incubate cells 36 to 64 h
- carefully remove the medium
- Washing steps: (250?l/well)

PBS	1x
MeOH	2x 5min
TCA	2x 10min
H_2O	1x

- lyse cells in 250?l 0.3M NaOH per well
- transfer 2.5 ml ECO Plus into the appropriate scintillation vials
- transfer cell lysate into the scintillation vials
- count by liquid scintillation (ß-counter; Beckmann Instruments)