

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

Material:

- 1% Gelatine in ddH₂O, 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
- ³H-Thymidine 1mCi/ml, 1:40 verdünnt in PBS (0,025mCi/ml)
- PBS, Methanol, 5% TCA, ddH₂O, 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 2×10^3 cells/well in basal medium (2.5% CS)
- incubate cells 7 to 14 d at 37°C and 5% CO₂
- stimulate the cells with the desired growth factors (as positive control 10µl CS is used)
- add 20µl ³H-Thymidine solution [0.025mCi/ml] per well
- incubate cells 36 to 64 h
- carefully remove the medium
- Washing steps: (250µl/well)

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| PBS | 1x |
| MeOH | 2x 5min |
| TCA | 2x 10min |
| H ₂ O | 1x |
- lyse cells in 250µl 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 (β-counter; Beckmann Instruments)