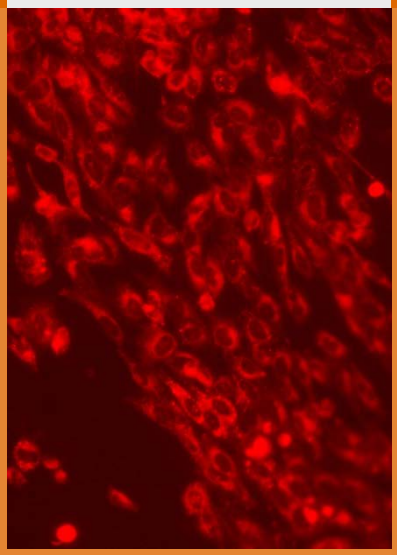
Staining of cells grown on fibrous collagen surfaces with the life cell tracking dye BTM DilC12(3)

To monitor living cells grown on the Collagen Cell Carrier® (CCC), Collagen Cell Carrier® „Ready-To-Use“ (RTU), or on/in Collagen Bio Tubes (CBT), life cell tracking fluorescent dyes like BDTM DilC12(3) may be used.

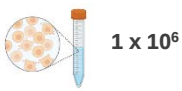
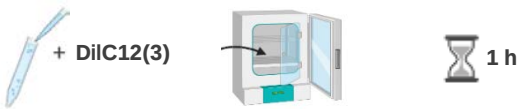
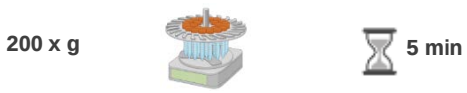
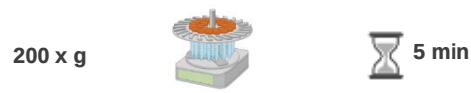
The optimal dye concentration and incubation time has to be optimized for every cell line or cell type according to the recommendations of the manufacturer. An (inverted) fluorescence microscope with a suitable filter set is required. Cells can be stained either prior to seeding or after cell attachment.



SaOs-2 cells, 4 days after seeding on CCC and stained with DilC12(3)

 | REVISION DATE: 09.04.2019

Exemplary staining protocol for SaOs-2 cells in suspension*

STEP 1	Adjust cell concentration from 2×10^4 to **1×10^6 cells per mL** in culture medium.	
STEP 2	**Add DilC12(3)** to a final concentration of **1.25 µg / mL**, mix and incubate at 37°C at 5% CO_2 for 1h.	
STEP 3	**Centrifuge** the cell suspension 5 min, 200 × g at room temperature.	
STEP 4	**Aspirate** the supernatant and **resuspend** the cell pellet in approx. **5mL PBS** to wash away excess fluorescent dye.	
STEP 5	**Centrifuge** cell suspension 5 min, 200 × g at room temperature.	
STEP 6	Aspirate the supernatant and **resuspend the cells** in the required volume of **culture medium**.	
STEP 7	Cells can be **seeded on fibrous collagen surface**, such as the CCC or Biotubes.	

Exemplary staining protocol for SaOs-2 cells adherent cell layer*

STEP 1

Prepare cell culture on the collagen product as described in the user protocol.

Continue with step 2 after attachment of cells to the collagen surface.

STEP 2

Dilute the fluorescent dye to a final concentration of 1.25 µg / mL DiIC12(3) in cell culture medium.

Prepare enough solution to cover all cell samples.
Warm the solution to room temperature.

prepare 1.25 µg /mL DiIC12(3)

RT

STEP 3

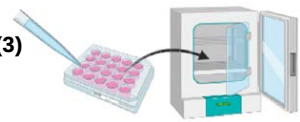
Wash the cells with PBS pre-warmed to room temperature to remove floating cells and aspirate supernatant.

wash 1x with PBS & aspirate

STEP 4

Add the prepared **staining solution**.
Incubate for 1 h at 37°C / 5% CO_2.

+ DiIC12(3)



1 h

STEP 5

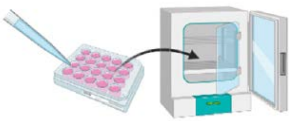
Aspirate supernatant.
Wash the cells **twice** with PBS pre-warmed to room temperature to remove excess of dye.

wash 2x with PBS & aspirate

STEP 6

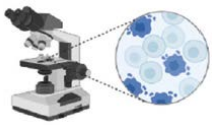
Aspirate PBS.
Add the required amount of **culture medium** and **continue cell culture** as usual.

+ culture medium



STEP 7

Monitor cells.
There is no decrease in metabolic cell activity and cells can be easily monitored for at least 6 population doublings



monitor for ≥ 6 population doublings

**All data and recommendations correspond to the present state of our knowledge; they are published without engagement. We reserve the right to make alterations and additions in line with technical developments without prior notice. The user is obliged to check whether our products meet with his own technical requirements. We are happy to answer any queries.*

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