Staining of cells grown on Fibrous Collagen Surfaces with the life cell tracking dye BDTM DiIC12(3)

Before starting please note:

To monitor living cells on the Collagen Cell Carrier (CCC), on Collagen Cell Carrier "Ready-To-Use" (RTU) or on / in Collagen Bio Tubes (CBT), life cell tracking fluorescing dyes like BDTM DiIC12(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.

Exemplary staining protocol for SaOs-2 cells:

Staining of a cell suspension:

1. Adjust the cell concentration from 2×10^4 to 1×10^6 cells per ml in culture medium
2. Add DiIC12(3) to a final concentration of 1,25 µg / mL, mix and incubate at 37°C for 1h
3. Centrifuge the cell suspension 5 min, 200 × g, 5 min at room temperature
4. Aspirate the supernatant
5. To wash away excess of fluorescent dye resuspend the cell pellet in the double volume PBS/2% FCS or culture medium relative of the used staining solution volume.
6. Centrifuge the cell suspension 5 min, 200 × g, 5 min at room temperature
7. Aspirate the supernatant and resuspend the cells in the required volume of culture medium
8. Cells can be seeded on Fibrous Collagen Surface

Viscofan BioEngineering
A Business Unit of Naturin Viscofan GmbH
Badeniastraße 13
69469 Weinheim
Germany

Tel.: +49 (0)6201 86-358
Fax: +49 (0) 6201 86-226
Email: sales@bio.viscofan.com
www.viscofan-bioengineering.com

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Staining of an adherent cell layer:

1. Prepare the Collagen product as described in the appropriate user protocol. Continue with step 2 after cells have attached to the collagen surface.

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 37°C.

3. To remove floating cells wash the cells with a suitable volume of PBS pre-warmed to at least room temperature.

4. Aspirate PBS and add the prepared staining solution. Incubate for 1 h at 37°C in the CO_2 incubator.

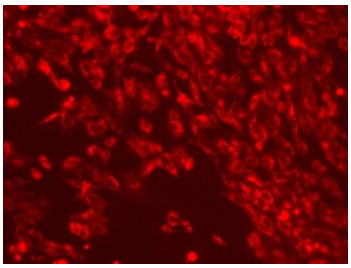
5. Aspirate the supernatant

6. To wash away excess of dye wash the cells twice with a required volume of PBS pre-warmed to at least room temperature.

7. Aspirate the PBS, add a required amount of culture medium and continue cell culture as usual.

No decrease in metabolic cell activity was observed and cells could be easily monitored for at least 6 population doublings.

SaOs-2 cells seeded on CCC and stained with DiIC12(3)



day 4 after seeding

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