BioResearch

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Determination of RAFT™ 3D Culture Viability Using the LIVE/DEAD® Assay

Technical Notes

Introduction

Assessing the viability of a RAFT™ 3D Cell Culture may not seem as trivial as assessing the viability of a 2D cell culture. However, we show in this technical note that by adding some basic controls, the LIVE/DEAD® Assay is a straightforward and reliable method to assess the viability of cells inside a RAFT™ 3D Cell Culture.

Materials / Methods

General Protocols

- RAFT™ Absorbers and reagent kits, visit www.lonza.com/raft for list of RAFT™ Products and RAFT™ Protocols
- LIVE/DEAD® Viability/Cytotoxicity Kit (Life Technologies)
- Widefield fluorescent microscope with appropriate filters
- RAFT™ Instructions downloadable from www.lonza.com/raft under 'product knowledge center'
- For a list of recommended manufacturer cell culture plates (either 96-well black wall or 24-well plate not supplied with the kit), contact Lonza scientific support team

Make RAFT™ Cultures as Indicated in the Protocol Supplied with the RAFT™ Kits.

To use the LIVE/DEAD® Assay Kit with RAFT™ Cultures, you can follow the "fluorescence microscopy protocol" provided with the LIVE/DEAD® Viability/Cytotoxicity Kit up to point 3.5.

However, we recommend the following adjustments:

1. To get a thorough wash of the whole 3D cell culture, the medium on the culture is aspirated and replaced with 100 µL PBS and the plate left for 5-10 minutes on a rocker (while preparing the combined LIVE/DEAD® Assay reagents)
2. To prepare the dead cells sample, we incubate the cells with 1%w/v saponin for at least 30 minutes. If you have added 100 µL of medium onto your RAFT™ Cultures, just add 25 µL of 5% w/v saponin 30 minutes before performing the assay
3. Due to the presence of the collagen matrix, it is advised to include acellular RAFT™ 3D Cultures to act as a control for background noise

Note: We have found that in the case of human dermal fibroblasts, final concentrations of 0.4 µM for Calcein AM and 4 µM for Ethidium homodimer 1 were optimal.

Instead of point 4.1 in the supplied LIVE/DEAD® Viability/Cytotoxicity kit protocol onward use the following protocol:

Aspirate the PBS added previously from the wells and replace it with 100 µL of the combined LIVE/DEAD® Assay reagents.

You can begin capturing images within 10 minutes of adding the reagents, however, keep in mind that the fluorescence emitted by Calcein AM will continue to increase in the live cells over time. Typically, we image the RAFT™ Culture between 10 minutes and 1.5 hours of adding the reagents.

Materials and methods used with human dermal fibroblasts

Early passage of human dermal fibroblasts (HDFn) were seeded into RAFT™ 3D Cell Cultures at a density of 3,000 cells/well of a 96-well plate, following the protocol supplied with the RAFT™ Kit. Two acellular cultures were also made for use as a background control in the LIVE/DEAD® Assay.

Immediately after removing the RAFT™ Plate from the RAFT™ Culture Plate, 100 µL of DMEM containing 10% sterile-filtered serum and 1% antibiotic-antimycotic mix was added on top of each (cellular and acellular) RAFT™ Culture.

In this experiment after an overnight (1 day) or 7 days culture of the cells in RAFT™ Plate, the LIVE/DEAD® Assay was carried out, using at least 1 dead control and 2 background controls (acellular cultures).

Each culture was then looked at under the microscope and z-series at 5 µm intervals were captured using a fluorescence microscope fitted with a z-focus drive. The number of live cells (stained with Calcein AM) and dead cells (stained with Ethidium homodimer 1) were counted from 3 separate images over 2 wells for the "live" samples.

The background noise for Ethidium homodimer 1 was counted in 2 acellular cultures ($bckgd_{EthD1}$ ① and $bckgd_{EthD1}$ ②).

The background noise for Calcein AM was assessed in the acellular cultures and in the dead control, however there was no visible background detected.

Therefore, for each image, the percentage of viability was calculated as follow:

$$\%viability_{d=1 \text{ or } d=7} = \frac{\text{Number of live cells}}{\text{Number of live + dead cells} - \text{average } (bckgd_{EthD1} \text{ ①\&②})}$$

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Results

In Figure 1, we show the typical images that can be obtained after culturing HDFn in RAFT™ 3D Cell Cultures for 1 and 7 days and staining the cultures with the combined LIVE/DEAD® Assay reagents. We also show in Figure 1 some examples of the Calcein AM and Ethidium homodimer 1 stain on an acellular construct and a dead control.
Figure 2 shows that the mean percentage viability of HDFn is 92% at day 1 and 94% at day 7 using the methods described above.
For comparison we have added the viability observed with the same cells cultured at the same time in a 2D planar environment, which is at 95% and 98% at day 1 and day 7 respectively.

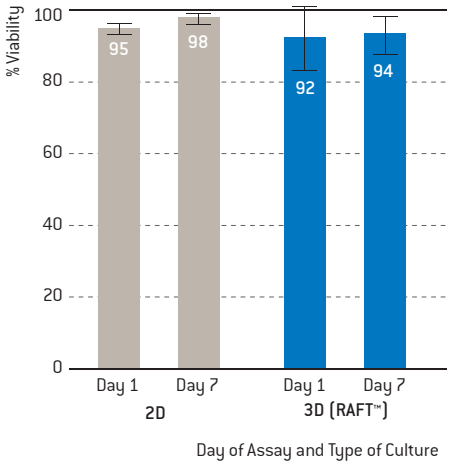

Figure 2: Comparison of the viability of HDFn after 1 and 7 days in a 2D or in a RAFT™ 3D cell culture. The percentage viability was determined as explained in the methods above and the average for 2 separate experiments is shown in this graph. The standard deviation is shown for each sample.

Conclusions

The LIVE/DEAD® Viability/Cytotoxicity Kit is an easy and rapid assay that can be used to assess cell viability in a RAFT™ 3D Cell Culture, provided that controls are included in the test to be able to take into account the possible background noise.
In this experiment, we show that HDFn display a mean viability between 92% and 94% when cultured in RAFT™ from day 1 and for at least 7 days, which is comparable to the viability observed for cells cultured in a 2D environment.

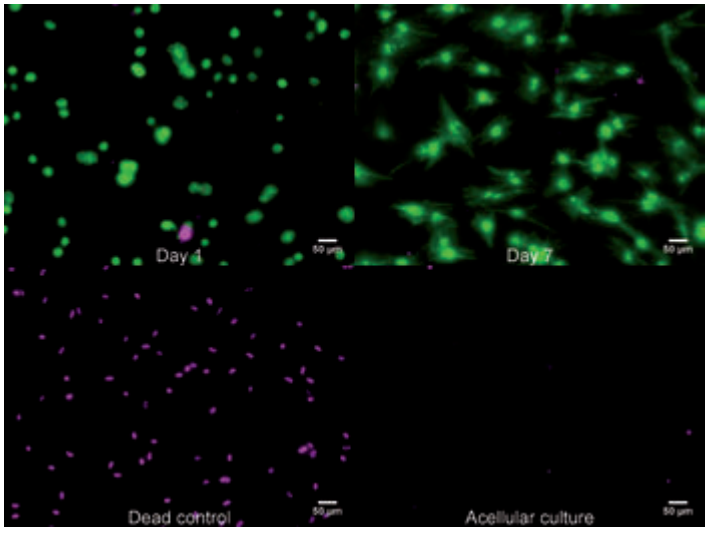

Figure 1: Examples of images that can be taken after staining RAFT™ Cultures with the combined LIVE/DEAD® Assay Reagents. HDFn live cells are displaying Calcein AM staining (green) while dead cells display Ethidium homodimer staining (magenta). Each image is a projection on the z-axis of a whole z-series.
Day 1 and Day 7: HDFn were cultured for 1 or 7 days respectively in RAFT™ Plate before being treated with the combined LIVE/DEAD® Assay reagents.
Dead control: HDFn cultured in RAFT™ Plate for 1 day were killed using 1% w/v saponin before being stained with the combined LIVE/DEAD® Assay reagents.
Acellular culture: RAFT™ Acellular Culture stained with the combined LIVE/DEAD® Assay Reagents.

This Technical Note was generated originally by TAP Biosystems, who developed the RAFT™ Products. Lonza is now the exclusive distributor of RAFT™ 3D Kits.

Contact Information

North America
Customer Service: +1 800 638 8174 (toll free)
order.us@lonza.com
Scientific Support: +1 800 521 0390 (toll free)
scientific.support@lonza.com

Europe
Customer Service: +32 87 321 611
order.europe@lonza.com
Scientific Support: +32 87 321 611
scientific.support.eu@lonza.com

International
Contact your local Lonza distributor
Customer Service: +1 301 898 7025
Fax: +1 301 845 8291
scientific.support@lonza.com

Lonza Cologne GmbH – 50829 Cologne Germany

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