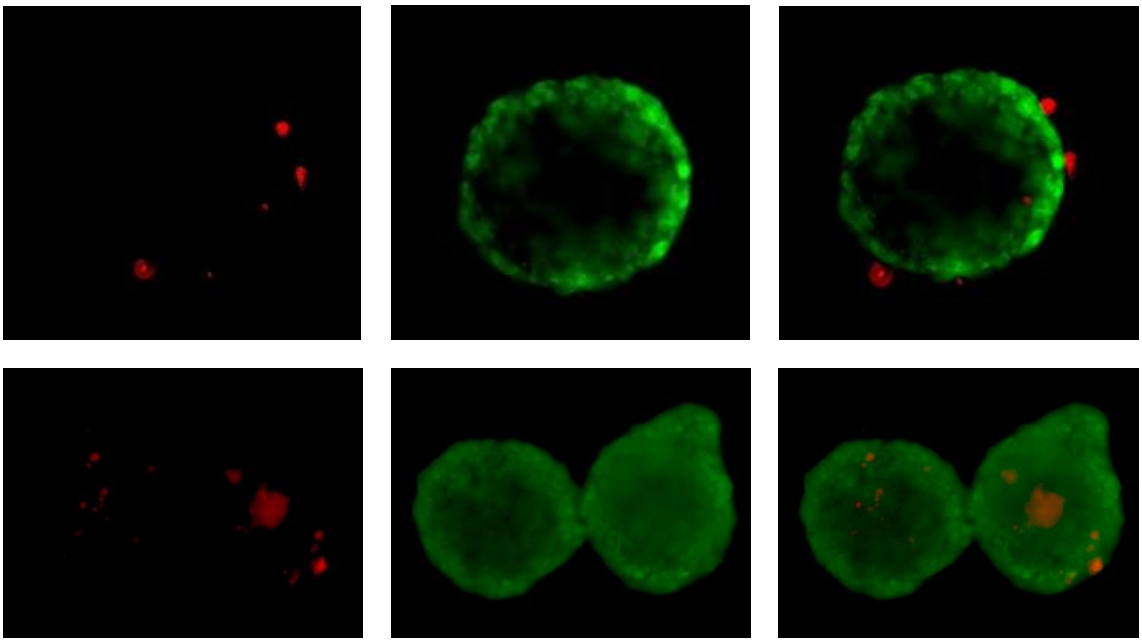
Application Note

Viability and Cytotoxicity Testing with 3D CoSeedis™



Introduction:

Testing 3D aggregates (such as spheroids or conical constructs) for their viability is an integral part to estimate their quality before they are processed any further. Likewise, cytotoxicity testing (e.g. upon compound treatment) may define an important step in assessing the susceptibility or resistance of the test cells to a certain treatment.
3D CoSeedis™ provides a validated method to distinguish live from dead cells by using the commercially available viability/cytotoxicity test from Molecular Probes, Inc. (LIVE/DEAD® Reduced Biohazard Viability/Cytotoxicity Kit #1; L-7013). This kit uses a two-colour fluorescence detection to assess animal cell viability. Living cells within an aggregate are emitting green fluorescence whereas dead cells appear red.

Procedure:

Material

- LIVE/DEAD® Reduced Biohazard Viability/Cytotoxicity Kit #1; L-7013; Molecular Probes, Inc.
- 3D constructs grown in the 3D CoSeedis™ matrix
- PBS with Mg^{2+}/Ca^{2+}
- 6-well plate (provided with 3D CoSeedis™ system)
- Fixative (e.g. 4% formaldehyde)

- Inverted fluorescent microscope

Protocol

1. Wash 3D CoSeedis™ matrices containing 3D constructs twice with 4-8 ml of PBS with Mg^{2+}/Ca^{2+} in a 6-well plate
   Note: only use 6-well plates that are recommended by abc biopply. For details, refer to the system protocols (https://biopply.com/products-applications/protocols/)
2. Allow the staining reagents (component A and B, see manufacturer's manual LIVE/DEAD® Reduced Biohazard Viability/Cytotoxicity Kit #1; L-7013) to warm to room temperature and shortly centrifuge the vials before opening
   Note: component A and B are in Dimethylsulfoxid (DMSO). Handle with appropriate care!
3. Staining stock solution to stain 3D CoSeedis™ matrix:
   a. 2 ml PBS with Mg^{2+}/Ca^{2+}
   b. 4 µl component A
   c. 4 µl component B

   Note: protect solutions from light!
4. Mix staining stock solution well before applying to 3D CoSeedis ™ matrix
5. Remove PBS with Mg^{2+}/Ca^{2+} from 3D CoSeedis™ matrix
6. Add 2 ml of the staining stock solution to an empty well of a 6-well plate
7. Transfer 3D CoSeedis™ matrix containing the 3D constructs
8. Quickly shake 6-well plate carefully
9. Incubate 6-well plate in the dark at room temperature for at least 15 minutes. Longer incubation may be required depending on the compactness of the 3D constructs
10. Remove staining stock solution and wash 3D CoSeedis™ matrix twice with 4 - 8 ml PBS with Mg^{2+}/Ca^{2+}
11. For immediate analysis, no fixation is required. However, for long-term storage or later analysis, fix 3D CoSeedis™ matrix in fixative
    Example for one 3D CoSeedis ™ matrix with 4% formaldehyde:
    Note: formaldehyde is toxic and carcinogen. Thus, handle with appropriate care!
    a. Remove PBS with Mg^{2+}/Ca^{2+} from 3D CoSeedis™ matrix
    b. Add 2 – 3 ml of 4% formaldehyde
    c. Incubate overnight at 4˚C (close 6-well plate tightly with Parafilm®)
    d. Remove formaldehyde and wash 3 times in PBS with Mg^{2+}/Ca^{2+}
    e. Long-term storage in PBS with Mg^{2+}/Ca^{2+} (close 6-well plate tightly with Parafilm®) at 4˚C
12. Use an inverted fluorescent microscope for the analysis. Filter settings are according to the manufacturer's manual (Molecular Probes, Inc.)

Additional information:

For additional information, please contact us under service@biopply.com or contact your local abc biopply partner.
Order information can be found at:
https://biopply.com/products-applications/order-3d-coseedis/
