Imaging V-Bottom Ultra-Low Attachment Plates For Organoid Generation Using CELLAVISTA®

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Tissues have a complex structure that cannot be fully represented in traditional two-dimensional (2D) cell culture systems. This is particularly significant in the field of drug discovery, where 2D models may not accurately predict the response of tissues to a particular compound. In response, three-dimensional (3D) cell culture systems have gained popularity, as they allow cells to interact in all three dimensions, providing a more representative model of *in vivo* tissue biology. One such 3D culture technique is organoid culture, in which cellular aggregates derived from primary tissues or stem cells self-organize into organotypic structures. [1], [2]

Among the precursors of organoids are embryoid bodies (EBs). EBs are the three-dimensional aggregates formed in suspension by pluripotent stem cells (PSC), including embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC). A common platform to generate specific cell lineages from PSCs is EB differentiation [3]. However, successful organoid differentiation depends on the ability to control the initial diameter of the EBs, as variations in diameter size can severely affect differentiation. [4]

A solution to this problem is provided by v-bottom plates with an ultra-low attachment (ULA) surface. The coating prevents attachment of the cells to the surface and the special geometry forces the formation of EBs of consistent size [2]. Their pointed bottom (depicted in Fig. 1) has been demonstrated to facilitate cell

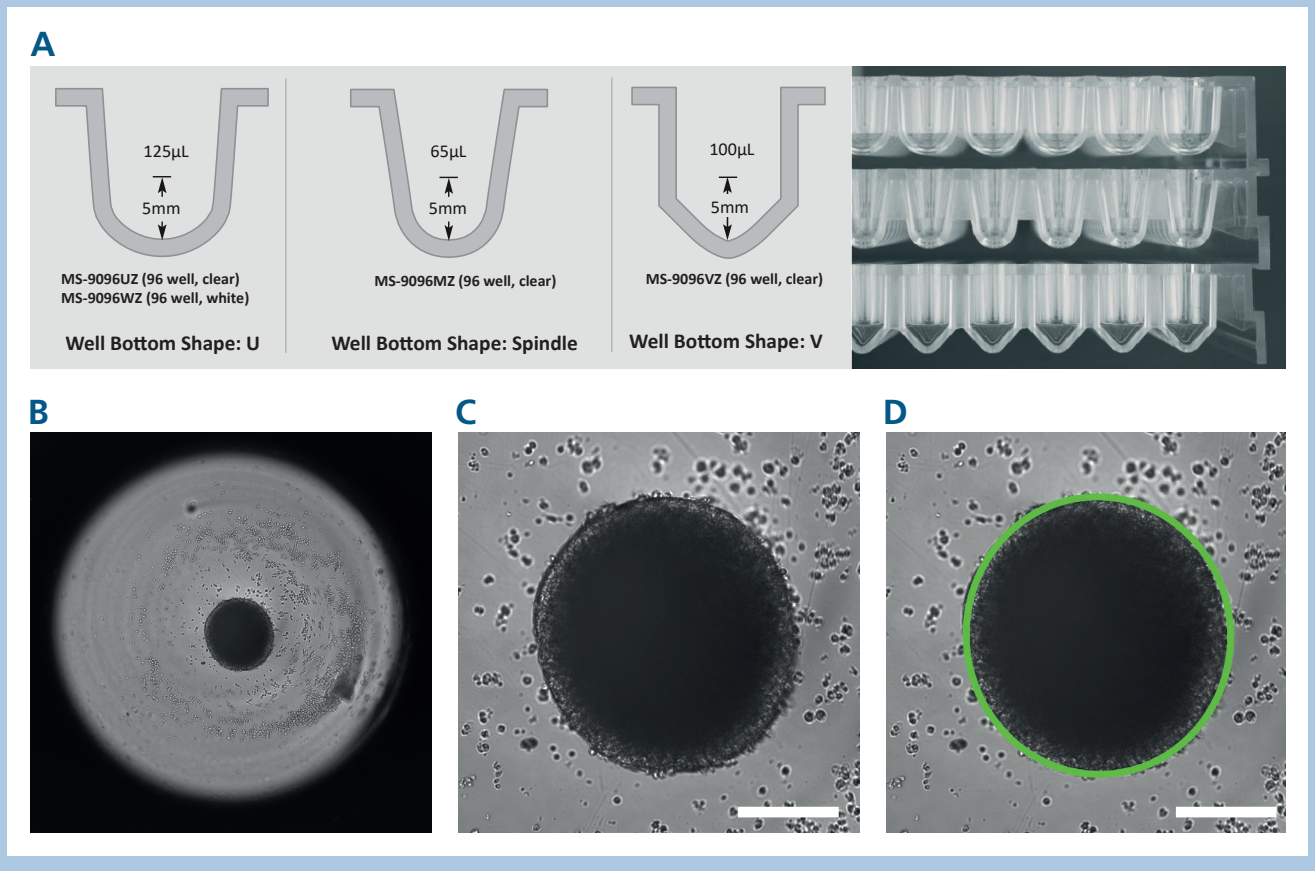

FIG. 1. EMBRYOID BODIES GROWN IN V-BOTTOM PLATES CAN ROBUSTLY BE ANALYZED USING SYNENTEC'S IMAGERS

A) Specifications of v-bottom plates (as described in the PHC Europe manual) B) Example image of a full well C) Same picture as in B but zoomed in (scale bar 200 µm) D) The **Spheroid Quantification** Application of YT-SOFTWARE detects the spheroid/EB (green circle).

aggregation more effectively than standard U-bottom plates [4]–[6]. These plates have been shown to generate EBs with consistent diameters and have become the standard for EB formation [2]. Consequently, several studies have reported the successful use of v-bottom plates for the generation of brain organoids [6]–[10].
However, imaging v-bottom plates can be challenging due to the special geometry of their plate bottom. Here we show that we have successfully imaged 96-well v-bottom plates using our high-content imagers. EBs were generated from iPSCs and grown in PrimeSurface® v-bottom plates from PHC. Fig.1 shows example pictures of an EB as the precursor for brain organoids. All EBs were in the same focal plane and could be imaged and quantified using the **Spheroid Quantification** application of our YT-SOFTWARE®. We imaged a defined focal plane (autofocus set to "never") to enable fast measurement and to avoid false focusing due to the special plate geometry. Imaging of a 96-well plate with three different focal planes (to ensure that EBs are still properly imaged if they vary in size) took approximately 1 min, ensuring a high throughput. This throughput can be enhanced by using our automation system, in which the robot SYBOT X-1000 can automatically transfer up to 42 plates from CYTOMAT™ 2 C-LiN incubator to our imagers NYONE® or CELLAVISTA® at regular intervals. Alternatively, our imagers can easily be integrated into robotic systems from various manufacturers. In summary, our products enable convenient high-throughput screening and monitoring of EBs or organoids grown in 96-well plates.

At the time of publishing, no more details regarding materials or methods can be described due to confidentiality of the data.

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