Application Note

INTEGRA

3D cell culture processing with the VIAFLO 96/384 handheld electronic pipette and the Corning® Elplasia® plate

Introduction

The use of spheroids and other 3D cell culture models in cancer research and drug discovery has grown in recent years, as these are more physiologically relevant than traditional 2D models. Corning Elplasia plates enable researchers to generate multiple spheroids in a scaffold-free model using microcavity technology, making it possible to obtain several high signal data points from a single well without increasing the spheroid size. However, 3D cell seeding and washing within the plate require very accurate pipetting in order to increase reproducibility.

A 96 or 384 channel pipette, such as the VIAFLO 96/384 handheld electronic pipette, can make working with 3D cell plates like Corning Elplasia much more efficient, reproducible and simple to perform, as parameters such as pipetting height and speed can be adjusted.

Key benefits:

- VIAFLO 96/384 handheld electronic pipette is simple to program, enabling exact control of both dispense and aspiration heights, as well as speed, to avoid damaging delicate cell spheroids
- VIAFLO 96/384 ensures reproducible liquid transfer into each individual well of the Elplasia plate for uniform spheroid formation
- Up to 2000 spheroids can be generated and cultured per plate under uniform conditions
- Setting the Tip Align strength and using the VIAFLO 96/384's Z-height function enables easy and accurate pipetting into the plate
- For even greater reproducibility, the VIAFLO 96/384 can be used in a hands-free automatic mode, ensuring tasks are performed the same way every time

Step-by-step procedure:

Experimental set-up

The cell lines HT-29/GFP (Creative Biogene CSC-RR0119), HepG2 (ATCC® HB-8065™), and A549 (ATCC® CCL-185™) were thawed and cultured following the vendors' protocols. Cells were harvested using 0.05 % trypsin with 0.53 mM EDTA (Corning 25-052-CV) and resuspended in the recommended culture media.

Overview of the 3D cell culture process

Step 1: Cell seeding
Step 2: Media change

VIAFLO 96/384

1. Cell seeding

STEP: Cell seeding into the Corning Elplasia 96 well plate with the VIAFLO 96/384 handheld electronic pipette

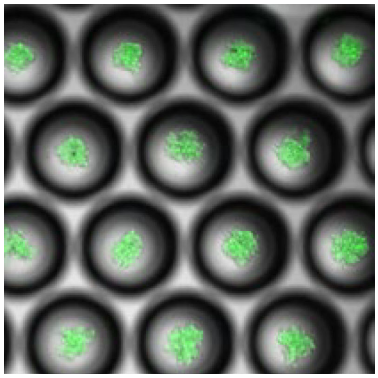

Figure 1: Seeding HT-29/GFP cells using a VIAFLO 96/384 handheld electronic pipette resulted in consistent, single spheroids in each microcavity. Representative image of HT-29/GFP cells seeded at 500 cells per microcavity in a 96 well Corning Elplasia plate. Image was taken with a CellInsight CX7 confocal imager using a 4x objective with one field digitally zoomed in.

HOW TO: Accurate handling of the cell suspension during seeding is critical to ensure that the spheroids are of uniform size across the entire microplate. A VIAFLO 96/384 handheld electronic pipette equipped with a 10 – 300 µl 96 channel pipetting head was used to pre-wet a 96 well Corning Elplasia plate with 50 µl per well of McCoy's 5A medium supplemented with 10 % fetal bovine serum.

The plate was centrifuged at 500 x g for 1 minute to remove air bubbles, and HT-29/GFP cells were then seeded with the VIAFLO 96/384, using 100 µl per well at 500 cells per microcavity. The plate was incubated for 1 day at 37 °C, 5 % CO_2 in a humidified incubator to allow spheroid formation.

Experimental results (Corning) showed low variability in spheroid size in each well across the entire microplate, thanks to the accurate dispensing of the cell suspension with the VIAFLO 96/384 (**Figure 1 and 2**).

Tips:

- The Z-height should be defined to provide more accurate results
- Adjust the handle sensitivity as required by the user for optimal automation of the process

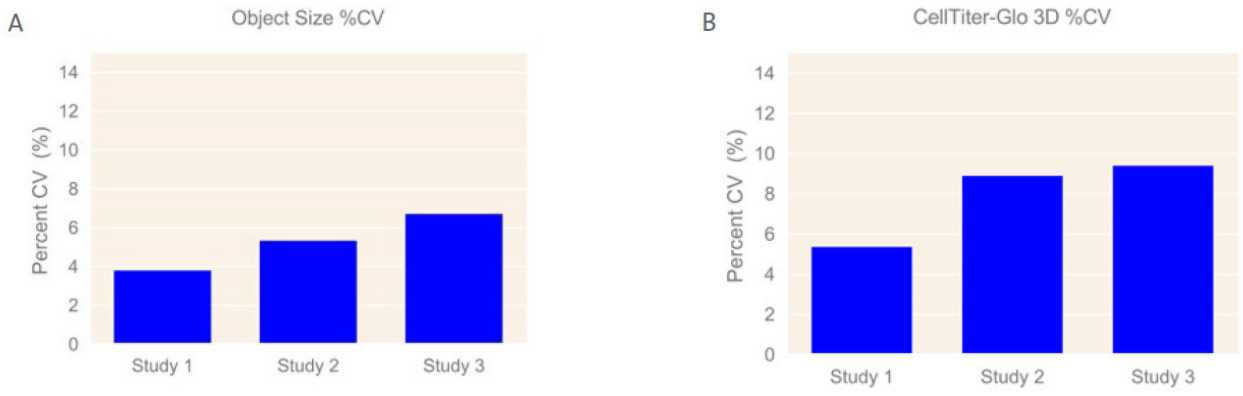

Figure 2: Low percent CV values (<10 %) were achieved for cell seeding using a VIAFLO 96/384 handheld electronic pipette. HT-29/GFP cells were seeded into a 96 well Corning Elplasia plate with a VIAFLO 96/384 on three different days (studies 1-3). (A) After overnight incubation, the average spheroid diameter (object size) in each well was determined using a CellInsight CX7 HCS platform and used to calculate a %CV value for each plate. (B) The same plate was then used for a CellTiter-Glo™ 3D luminescence assay, and the resulting luminescence was used to calculate a %CV value for each plate.

2. Media exchange and washing steps

STEP: Changing cell culture media and washing the cells without disturbing the spheroids

HOW TO: The VIAFLO 96/384 handheld electronic pipette was used to pre-wet the plate with 50 µl of culture media, which was then centrifuged at 500 x g for 1 minute to remove trapped air prior to cell seeding. Three different cell lines (HT-29/GFP, HepG2 and A549) were seeded using 100 µl per well at seeding densities of 125, 250, 500 and 1000 cells per microcavity (one column per density). The plate was incubated in a 37 °C, 5 % CO_2 humidified incubator for 1 day.

The next day, the VIAFLO 96/384 was used to add 20 µl of Hoechst 34580 to the plate wells to stain the cell nuclei, followed by incubation in a 37 °C, 5 % CO_2 humidified incubator for 30 minutes.

The plate was then washed using the VIAFLO 96/384. The culture medium was removed, leaving a volume of 50 µl per well, and 150 µl per well of DPBS without calcium and magnesium was added. Finally, the plate was washed 4 times and imaged after each wash to monitor spheroid loss.

The experimental results (Corning) show the ability of the VIAFLO 96/384 handheld electronic pipette to perform high quality media exchange and washing steps (**Figure 3 and 4**).

Tip:

- By using the 10 – 300 µl 96 channel pipetting head with the VIAFLO 96/384 throughout, cell seeding can be performed faster without needing to change the pipetting head

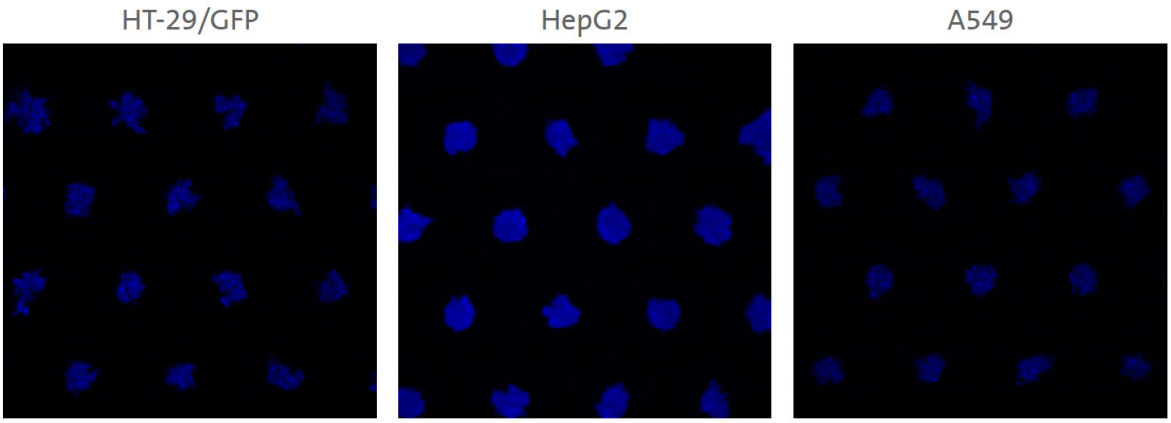

Figure 3: Three cell lines formed consistent, single spheroids in each microcavity of a 96 well Corning Elplasia plate. Representative images of nuclei-stained HT-29/GFP, HepG2, and A549 cells seeded at 500 cells per microcavity in a 96 well Corning Elplasia plate. Images were taken with a CellInsight CX7 confocal imager using a 4x objective with one field digitally zoomed in.

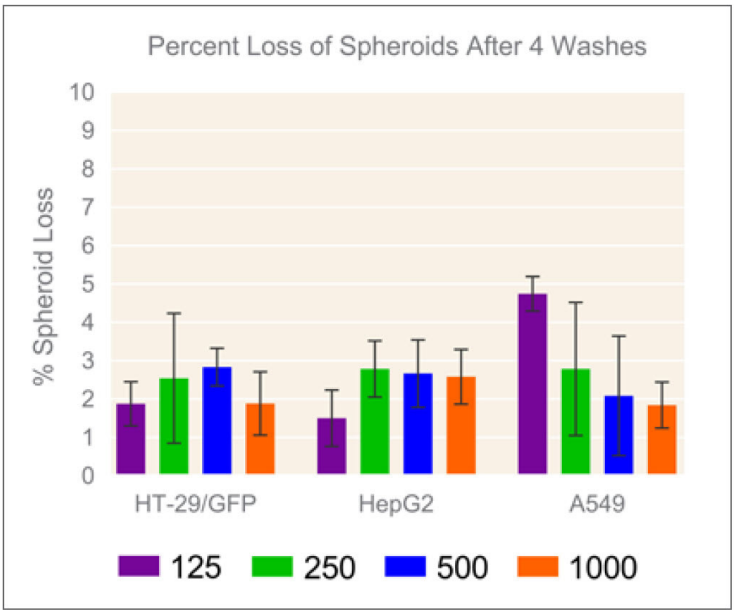

Figure 4: Four washes with the VIAFLO 96/384 handheld electronic pipette resulted in less than 5 % spheroid loss from 96 well Corning Elplasia plates. The HT-29/GFP, HepG2, and A549 cell lines were seeded at 125 to 1000 cells per microcavity and incubated overnight. A VIAFLO 96/384 was used to add Hoechst stain to each well and to perform 4 wash steps, removing all but 50 µl/well and then adding 150 µl/well of DPBS. Each plate was imaged using a CellInsight CX7 to count the number of spheroids per well before and after each wash. Data shown with standard error of the mean from three independent studies. N=24 wells.

Conclusion

- Using the VIAFLO 96/384 handheld electronic pipette resulted in low variability in spheroid size, with %CVs <10.
- The VIAFLO 96/384 settings allow efficient media exchange and washing as the pipette tips are always set to the same location and height to disturb the spheroids as little as possible, minimizing spheroid loss.
- The VIAFLO 96/384 simultaneously dispenses into all channels to ensure robust production of highly reproducible uniform spheroids across the entire microplate.
- Improved speed of plate filling compared to traditional, manual multichannel pipettes.
- The small footprint of the VIAFLO 96/384 enables use in a laminar flow cabinet.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	6001/6031	VIAFLO 96/384 handheld electronic pipette	https://www.integra-biosciences.com/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA Biosciences	6103	96 channel pipetting head 10 – 300 µl	https://www.integra-biosciences.com/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA Biosciences	6435	300 µl Sterile, Filter GripTips	https://www.integra-biosciences.com/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	6307	300 ml automation friendly Clear Advantage™ reservoirs (polystyrene)	https://www.integra-biosciences.com/en/reagent-reservoirs/automation-friendly-reagent-reservoirs

Manufacturer	Part Number	Description	Link
Corning	4442	Corning Elplasia 96-well round bottom plate, ULA surface	https://www.corning.com/worldwide/en/products/life-sciences/products/microplates/elplasia-plates.html
Corning	10-050-CV	McCoy's 5A	https://ecatalog.corning.com/life-sciences/b2b/US/en/Media,-Sera,-and-Reagents/Classical-Media/McCoy's-5A-(Iwakata-&-Grace-Mod-)/Corning%C2%AE-McCoy%E2%80%99s-5A-(Iwaketa-and-Grace-Modification),-1X/p/10-050-CV
Corning	25-052-CV	Trypsin EDTA 1x	https://ecatalog.corning.com/life-sciences/b2b/US/en/Media,-Sera,-and-Reagents/Cell-Culture-Reagents/Enzymatic-Cell-Dissociation-Reagents/Corning%C2%AE-Trypsin/p/25-052-CV
Corning	35-010-CV	Fetal bovine serum	https://ecatalog.corning.com/life-sciences/b2b/US/en/Media,-Sera,-and-Reagents/Animal-Sera/Animal-Sera/Fetal-Bovine-Serum/p/35-010-CV
Creative Biogene	CSC-RR0119	HT-29 cell line	https://www.creative-biogene.com/GFP-Stable-Cell-Line-HT-29-CSC-RR0119-1271450-13.html
ATCC®	HB-8065™	Hep G2 cell line	https://www.lgcstandards-atcc.org/products/all/HB-8065
ATCC®	CCL-185™	A549 cell line	https://www.lgcstandards-atcc.org/products/all/CCL-185
Thermo Fisher Scientific	H21486	Hoechst 34580	https://www.thermofisher.com/order/catalog/product/H21486#/H21486

INTEGRA Biosciences AG
7205 Zizers, Switzerland
T +41 81 286 95 30
F +41 81 286 95 33
info@integra-biosciences.com

INTEGRA Biosciences Corp.
Hudson, NH 03051, USA
T +1 603 578 5800
F +1 603 577 5529
info-us@integra-biosciences.com

INTEGRA Biosciences Deutschland GmbH
35444 Biebertal, Deutschland
T +49 6409 81 999 15
F +49 6409 81 999 68
info-de@integra-biosciences.com

INTEGRA Biosciences SAS
95062 Cergy-Pontoise Cedex 1, France
T +33 (0)1 34 30 76 76
F +33 (0)1 34 30 76 79
info-fr@integra-biosciences.com

INTEGRA Biosciences Ltd.
Egham, Surrey TW20 9EY, UK
info-uk@integra-biosciences.com