Application Note

Semi-automated medium exchange in Akura™ 96 and 384 Spheroid Microplates with the VIAFLO 96 and VIAFLO 384

Introduction

3D human in vitro cell models are gaining increased importance in the drug discovery and development cycle of the pharmaceutical industry as they mimic the human *in vivo* responses of newly developed substances more precisely than 2D models. They can help to bridge the gap between the traditional 2D in vitro models and whole-animal systems as the traditional models do not effectively recapitulate the more complex signalling and cellular interactions found in vivo. Spheroids are scaffold-free 3D cell culture models that are aggregated from one or more organ-specific cell types.

InSphero has developed 3D cell-based assay solutions in the fields of liver toxicology, metabolic diseases, and oncology. Industrial use of these complex in vitro systems requires a perfect match of the tissue model, cell culture plates and liquid handling instrumentation. InSphero's Akura™ 96 and 384 Spheroid Microplates have been engineered for the generation, long-term cultivation, observation and testing of spheroids in 96 and 384 well formats in a simple, flexible, and automation-compatible platform. Its scalable format requires liquid handling systems for convenient, yet quick, reliable, and precise medium exchange, sampling, dosing, and spheroid transfer from multiple plates. The semi-automated VIAFLO 96 and VIAFLO 384 electronic pipettes are ideal for these routine lab tasks, offering 96 and 384 channel pipetting in a compact and easy-to-use platform, for increased productivity.

Key benefits:

- The ultra-low attachment Akura 96 and 384 Spheroid Microplates allow convenient scaffold-free formation of spheroids – one per well – below 750 µm via cellular self-assembly.
- The special well design featuring the SureXchange™ tapered ledge enables an easy medium exchange and near-complete removal in a single pipetting step, preventing spheroid loss and increasing yield during long-term spheroid growth and analysis.
- The 1 mm diameter flat-bottom observation window enables simple spheroid observation, and a greater distance between the observation windows of adjacent wells reduces well-to-well imaging crosstalk compared to standard 96 and 384 well plates.
- An accurate pipetting system, such as the VIAFLO 96 and VIAFLO 384, is crucial to ensure seeding of the same number of cells into all wells throughout the plate and produce highly uniform spheroid models. It also enables the addition of the correct concentrations of test compounds and guarantees an equal medium exchange between different wells to ensure responses with a high reproducibility.
- Several user-defined position settings are available for delicate pipetting operations such as medium exchange. The Z height can be set to ensure that all subsequent operations are performed at the same height and the same amount of medium is removed without inadvertently aspirating the spheroid. The pipette tip can be precisely positioned above the spheroid for a reliable transfer without accidental damage.
- A very helpful feature of INTEGRA's VIAFLO 96 and VIAFLO 384 is the wide range of adjustable pipetting speeds. This ensures a safe medium exchange without the risk of strong turbulences that could cause spheroids to be damaged by shearing forces or to float.

Application Note

INTEGRA

Overview: How to perform medium exchange in Akura 96 and 384 Spheroid Microplates with VIAFLO 96 and VIAFLO 384

Step-by-step procedure

Experimental set-up

The VIAFLO 96 and VIAFLO 384 are used for medium exchange in Akura 96 and 384 plates. The liquid handling steps are optimized to ensure there is no spheroid loss. The VIAFLO 96 is equipped with a 96 channel pipetting head (10-300 µl) and both spring loaded and standard plate holders. 300 µl Sterile, Filter GripTips are used. The spheroids are cultured in Akura 96 plates with 70 µl of medium. The VIAFLO 384 is set up with a 384 channel pipetting head (5-125 µl) and both spring loaded and standard plate holders. 125 µl Sterile, Filter GripTips are used. Spheroids are cultured in Akura 384 plates with 50 µl of medium. Customized VIALINK programs are provided for every step of the protocol.

VIAFLO 96

Program 1: Medium aspiration – Akura 96
Program 2: Medium dispensing – Akura 96
Program 3: Medium aspiration – Akura 384
Program 4: Medium dispensing – Akura 384

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1. Medium aspiration – Akura 96

STEP: Medium is aspirated from four Akura 96 plates and discarded or sampled

HOW TO: Load 300 µl Sterile, Filter GripTips onto the VIAFLO 96. Place a 300 ml automation friendly reagent reservoir on position B. Place an Akura 96 lid with the opening facing down on position A. When fixing the lid in position, make sure that its orientation notch does not face the spring of the plate holder (**see video**). Place the Akura 96 plate containing the spheroids onto the lid (**see video**). This allows the plate position to be manually adjusted to create a small horizontal offset (1-2 mm) with respect to the tips (**Figure 1a and Figure 2**). This ensures that the pipette tips are positioned on the SureXchange ledge of the well, preventing unintended aspiration of spheroids.

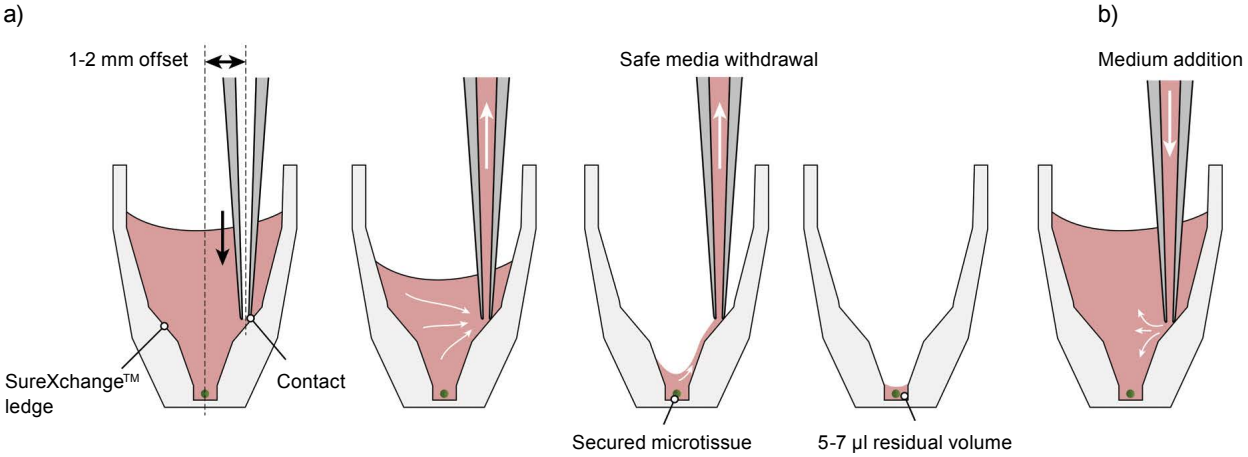

Figure 1: a) Correct pipette tip positioning on the SureXchange ledge of an Akura 96 plate during medium removal, and b) medium addition.

Select the custom program 'M_ASP_96_MAN'. The pipette aspirates 75 µl of medium at speed 1. This low speed minimizes the shearing force that the spheroid could be subjected to. The aspiration volume is set higher than the volume of the well (70 µl) to ensure complete removal of the medium. The special design of the well protects the spheroids and leaves a defined residual volume of 5-7 µl. This step is repeated 3 times, changing the plate containing the spheroids in between each step. When the last aspiration step is finished, move the pipette head to position B. A prompt on the pipette screen informs the user that the medium needs to be purged to the waste reservoir or into a collection plate. The purge step is performed with speed 8. Eject and discard the tips afterwards. The last step in the program is a loop, which means that the 4 aspiration steps and the final purging step are repeated up to 100 times, allowing processing of up to 400 plates. Abort the program once all the plates have been processed.

If the medium is intended to be sampled for downstream analysis, it must be dispensed after each aspiration step into a respective sampling plate.

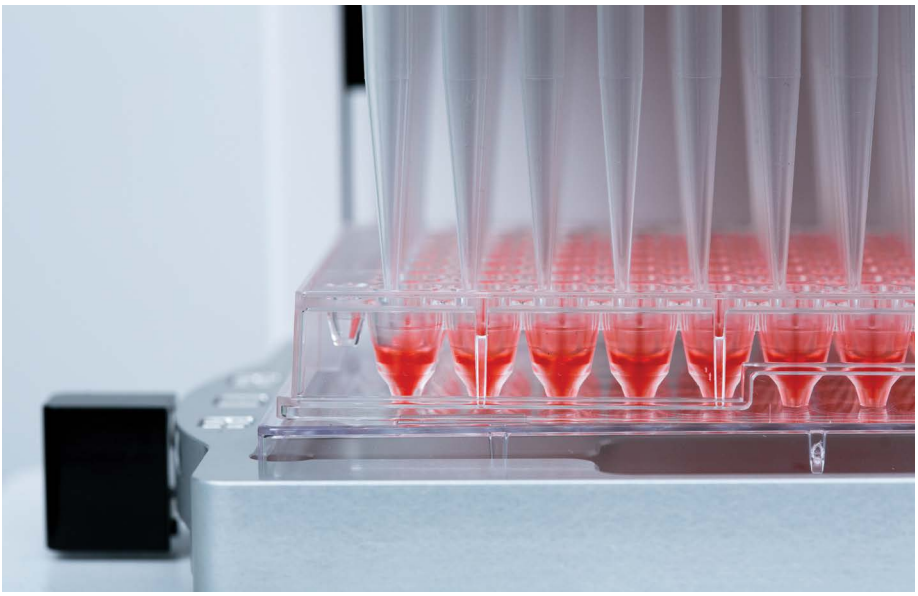

Figure 2: Horizontal offset of the Akura 96 plate on the VIAFLO 96 with respect to the tips.

Tips:

- Use Sterile, Filter GripTips for contamination-free processing.
- The Run button is pressed twice to purge the liquid when waste disposal is required. This warning step supports the user and makes sure that the waste is not dispensed back into the wells and is an important feature when exchanging the medium on several plates at once.

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2. Medium dispensing – Akura 96

STEP: Fresh medium is dispensed into the wells of four Akura 96 plates from the reservoir

HOW TO: Discard the used tips and load new 300 µl Sterile, Filter GripTips onto the VIAFLO 96. Place an automation friendly reagent reservoir with fresh medium on position B. Allow up to 10 ml of extra medium to prevent aspiration of air.

Place an Akura 96 plate onto an Akura 96 lid in position A. Adjust the plate manually to create a small horizontal offset (1-2 mm). Select and run the custom program 'M_DISP_96_MAN'. Move the pipette head to the reservoir on position B. The pipette aspirates 290 µl of fresh medium, which is sufficient to fill four plates with 70 µl medium plus 10 µl of excess volume. Move the pipette head to position A and gently immerse the pipette tips into the wells of the Akura 96 plate until the minimum height is reached (**Figure 1b**).

The pipette dispenses 70 µl medium into each well at a slow speed. The dispensing step is repeated 3 times, changing the plate after each step. The pipette will prompt the user to purge the medium that remains in the tip. The aspiration and dispensing process of four plates is repeated up to 100 times due to a loop at the end of the program.

Tip:

- Low pipetting speed can be set up in the VIALINK program to avoid damaging the delicate spheroids.

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3. Medium aspiration – Akura 384

STEP: Medium is aspirated from one or more Akura 384 plates and discarded or sampled

HOW TO: Load 125 µl Sterile, Filter GripTips on the VIAFLO 384. Place an empty 300 ml automation friendly reagent reservoir on position B.
Place an Akura 384 lid with the opening facing down on position A. To fixing the lid in position, make sure that the orientation notch of the lid does not face the spring of the plate holder (**see video**). Place the Akura 384 plate containing the spheroids onto the lid (**see video**). This allows the plate to be manually adjusted towards the user (‘Bottom of plate’, **Figure 3**), creating a small horizontal offset (1-2 mm) with respect to the tips. This ensures that the pipette tips are positioned on the SureXchange ledge of the well, preventing unintended aspiration of spheroids (**Figure 4a**).

Select the custom program 'M_ASP_384_MAN'. The pipette aspirates 70 µl at speed 1. This low speed minimizes the shearing force that the spheroid could be subjected to. The aspiration volume is set higher than the volume of the well (50 µl) to ensure complete removal of the medium. The special design of the well protects the spheroids and leaves a defined residual volume of 2-3 µl. A prompt on the pipette screen informs the user that the medium needs to be purged to the waste reservoir or into a collection plate. The last step in the program is a loop, which means that the required pipetting steps can be easily repeated for multiple plates without the need to restart the program. Abort the program, eject, and discard the tips once all the plates have been processed.

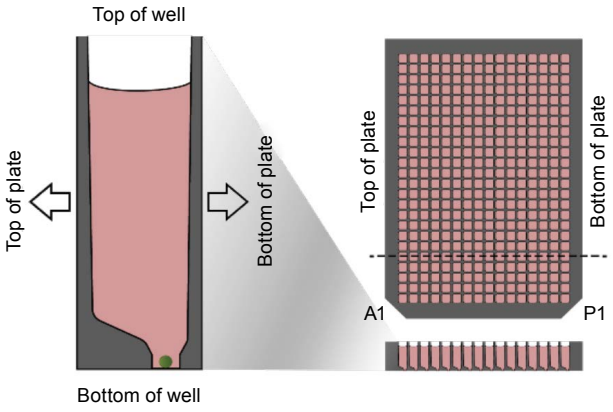

Figure 3: Well design of the Akura 384 plate showing the SureXchange ledge.

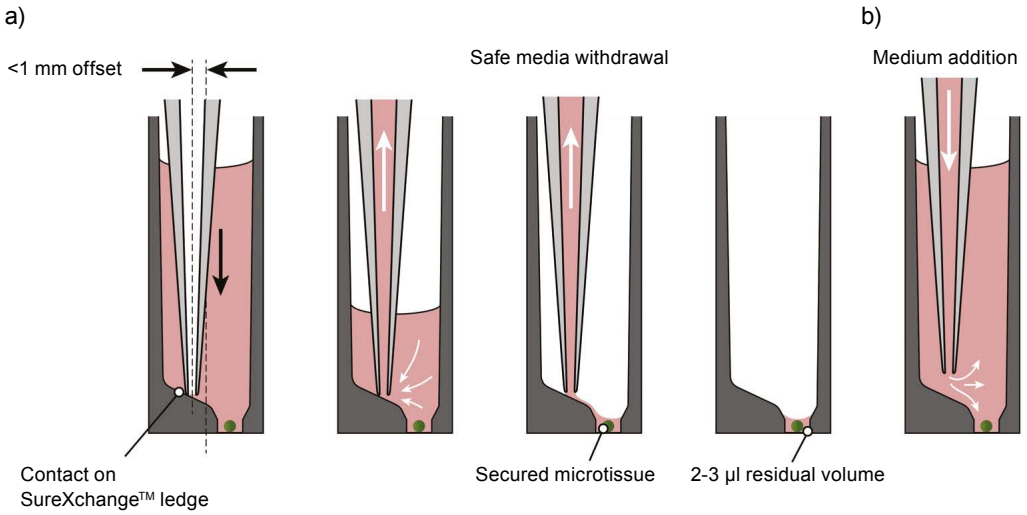

Figure 4: Correct pipette tip positioning on the SureXchange ledge of an Akura 384 plate during a) medium removal and b) medium addition.

4. Medium dispensing – Akura 384

STEP: Fresh medium is dispensed into two Akura 384 plates from the reservoir

HOW TO: Discard the used tips and load new 125 µl Sterile, Filter GripTips onto the VIAFLO 384. Place an automation friendly reagent reservoir with fresh medium on position B. Allow up to 10 ml of extra medium to prevent aspiration of air.

Place an Akura 384 plate onto an Akura 384 lid in position A. Adjust the plate manually towards the user (‘Bottom of plate’) to create a small horizontal offset (1-2 mm). Select and run the custom program 'M_DISP_384_MAN'. Move the pipette head to the reservoir on position B. The pipette aspirates 110 µl fresh medium, which is sufficient to fill two plates with 50 µl medium plus 10 µl of excess volume. Move the pipette head to position A and gently immerse the pipette tips into the wells of the Akura 384 plate until the minimum height is reached (**Figure 4b**). The pipette dispenses 50 µl medium into each well at slow speed. The dispensing step is repeated once more for a second well plate. The pipette will prompt the user to purge the medium that is remaining in the tip. The dispensing process for two plates is repeated up to 100 times due to a loop at the end of the program.

Tip:

- Z heights may vary from system to system, depending on the calibration. Check the correct height using a dummy plate. Pipette tips need to be positioned 0.2-0.3 mm above the spheroid. Lower pipette positioning could damage the spheroids.

Results

Three independent experiments showed <1 % spheroid loss from Akura 384 plates during nine consecutive medium exchanges (**Figure 5**).

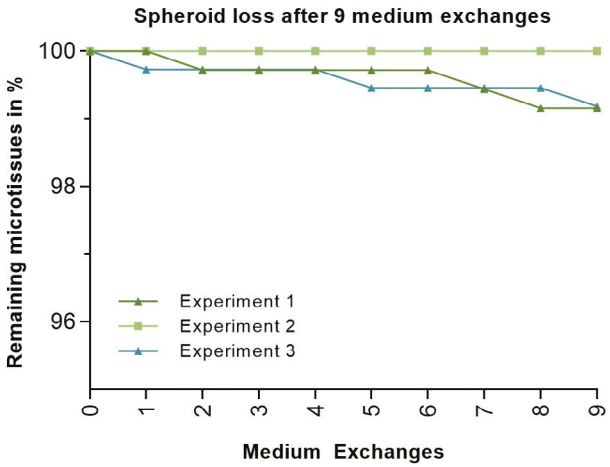

Figure 5: Nine consecutive medium exchanges were performed with InSphero's Human Islet Spheroids in Akura 384 plates using INTEGRA's VIAFLO 384. Three independent experiments aspirating >90 % of the medium showed <1 % spheroid loss.

Remarks

Partial plates: Programs can be adapted to a different number of samples at any time, giving laboratories total flexibility to meet current and future demands.

Automatic mode: The VIAFLO 96 and VIAFLO 384 handheld electronic pipettes can operate in an automatic mode, ensuring that all the liquid handling steps are performed in the same way. The customized automatic programs can be found in the download section. **IMPORTANT:** When using the automatic programs there is no need to place the lid below the Akura 96 and 384 plates.

Spheroid collection and transfer: The special coating of the Akura plates prevents adherence of the spheroids to the bottom of the well. This aids the collection of microtissues for transfer into another plate format, or for further processing. The customized program for spheroid collection and transfer (TRANS_TO_384) is available in the download section.

Conclusion

- The VIAFLO 96 and VIAFLO 384 handheld electronic pipettes ensure precise and accurate medium exchange in Akura 96 and 384 plates with minimal spheroid loss.
- Setting the Z height and the Tip Align on the VIAFLO 96 and VIAFLO 384 operator-induced pipetting minimizes variability.
- The motor-assisted movements of the VIAFLO 96 and VIAFLO 384 reduce strain on the arms and wrists during pipetting of multiple plates.
- The compact design of the VIAFLO 96 and VIAFLO 384 fits into most laminar flow cabinets when sterile conditions are required.
- GripTips deliver a precise and consistent tip seal. This ensures that all tips on a multichannel pipette are at exactly the same height – increasing the accuracy and precision of each multichannel dispense – and never accidentally fall off.
- The time to result can be drastically reduced when using the 96 or 384 channel pipettes.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA	6001	VIAFLO 96 base unit	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA	6031	VIAFLO 384 base unit	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA	6215	Spring loaded plate holder A	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA	6205	Standard plate holder	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA	6103	96 Channel Pipetting Head 10-300 µl	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA	6132	384 Channel Pipetting Head 5-125 µl	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384
INTEGRA	6327	300 ml Reservoir, Individually Sealed, Sterile, Polypropylene	https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
INTEGRA	6435	Sterile, Filter GripTips for 96 Channel Pipetting Heads	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
INTEGRA	6465	Sterile, Filter GripTips for 384 Channel Pipetting Heads	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
InSphero		Akura 96	https://insphero.com/shop-plates-Akura96
InSphero		Akura 384	https://insphero.com/shop-plates-Akura384

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