Application Note

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Automating media exchange and sample collection using Ronawk's Bio-Blocks® In Similare technology and the ASSIST PLUS

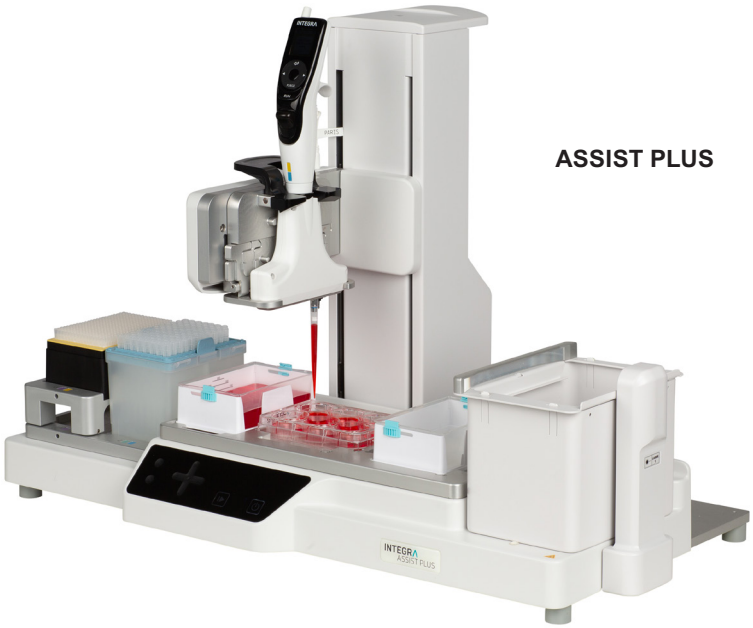
Introduction

Combining Ronawk's Bio-Block 3D hydrogel with INTEGRA's ASSIST PLUS pipetting robot and D-ONE pipetting module enables scalable extracellular vesicle (EV) and secretome production and collection. Previous studies with the Bio-Block system demonstrate how its design eliminates subculturing and provides a tissue-mimetic environment, where mesenchymal stromal cells retain their stem-like phenotype with enhanced viability, and significantly increase EV secretion. Automating media handling and secretome collection with the ASSIST PLUS and D-ONE ensures reproducibility and hands-free operation, streamlining benchtop scale-up of EV/secretome production.

Key benefits:

- Save media, space and plasticware by using 4 interlocking Bio-Blocks with a total culture area equivalent to 24 T-75 flasks, a fraction of the footprint and media volume needed for conventional cell culture.
- Precise and error-free pipetting with the ASSIST PLUS pipetting robot streamlines repetitive, error-prone liquid handling tasks, such as media exchange, and maximizes walk-away time.
- The D-ONE pipetting module enables accurate single-channel pipetting over a large volume range (5-1,250 µl), supporting many complex workflows.
- Streamlined experimental configuration using the readily adjustable CSV file (in the download section) ensures cGMP/GLP traceability and enables on-the-fly scalability, whether running just 3 wells or all 6.

Overview: How to perform media exchange and preservation in Bio-block 3D cell culture using the ASSIST PLUS and D-ONE Pipetting Module



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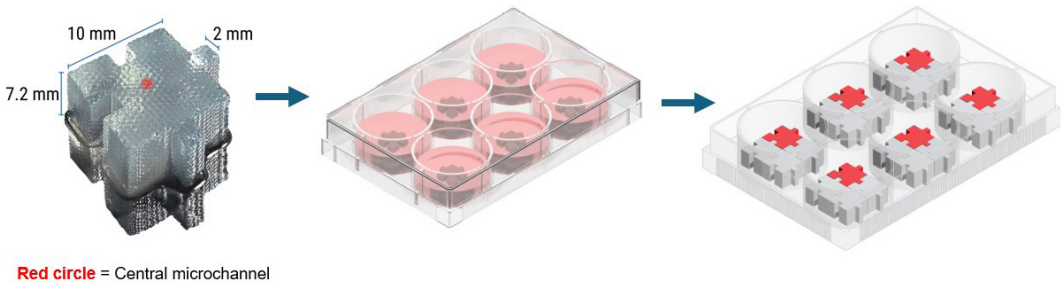

Figure 1: Modular Bio-Block 3D culture system enables passage-free expansion and enhanced EV yields. 4 interlocking hydrogel Bio-Blocks per well in a standard 6 well plate support cell growth in minimal media, preserving viability and reducing senescence through efficient nutrient diffusion. Integrating the Bio-Blocks modular culture system with fully automated media exchange and sample aliquoting ensures uniform, error-free handling, reduces reagent use and plastic waste, and enables rapid scaling for biomanufacturing.

When cell medium exchange is required, the ASSIST PLUS with D-ONE 5-1,250 µl single channel pipetting module is first used to collect spent medium from the culture plate. Each sterile 1,250 µl GRIPTIPS® pipette tip is positioned to avoid contact with the Bio-Blocks in the well, and the spent medium can be aliquoted into vials for downstream analysis if required. Fresh medium is then added to each well. The program provided for this application includes a labware change step, demonstrating the versatility of the ASSIST PLUS specifically when performing workflows that require multiple labware types.

Step-by-step procedure

Step 1: **Medium exchange**

How to: Load the 5-1,250 µl D-ONE single channel pipetting module and 50-1,250 µl sterile GRIPTIPS onto the ASSIST PLUS. Place a 300 ml automation friendly reagent reservoir holding clean medium on deck position A, the 6 well culture plate holding Bio-Blocks with cell culture on deck position B in portrait orientation, and an empty automation friendly reservoir on deck position C.

Select the program 'Bio-Block Medium Exchange' on the pipette. The pipette will aspirate 5,000 µl from each well of the plate using 4 separate aspirations of 1,250 µl each. To avoid damaging the Bio-Blocks with cells, the pipetting location is offset so the tip enters the culture plate away from the Bio-Blocks. Tip travel is activated so the tip travels dynamically during the aspiration, avoiding large air aspirations. Following each aspiration, the spent medium is dispensed into the reservoir on deck C. The reservoir can be exchanged after each well if the medium from each well needs to be analyzed separately. 1 pipette tip is used for each well, minimizing consumable costs for the user while avoiding cross contamination between wells.

When the medium removal is complete, a new tip is loaded onto the pipette, and addition of clean medium commences. Similar pipette tip offsets are used in this step to avoid damaging the Bio-Blocks. If all six wells do not contain the same cell type, and the medium needs to be processed separately, exchange the right most reservoir with a fresh insert to avoid cross contamination.

Tips:

- Use sterile, filter GRIPTIPS for contamination-free processing
- INTEGRA's ASSIST PLUS programming software, VIALAB, allows placement of the pipette tip anywhere within the source or target well. With Bio-Blocks, the pipetting position is set to avoid touching the hydrogel during addition or removal of media
- VIALAB software allows the operator to define tip changes, reducing waste when the same tip may be used for multiple liquid transfers

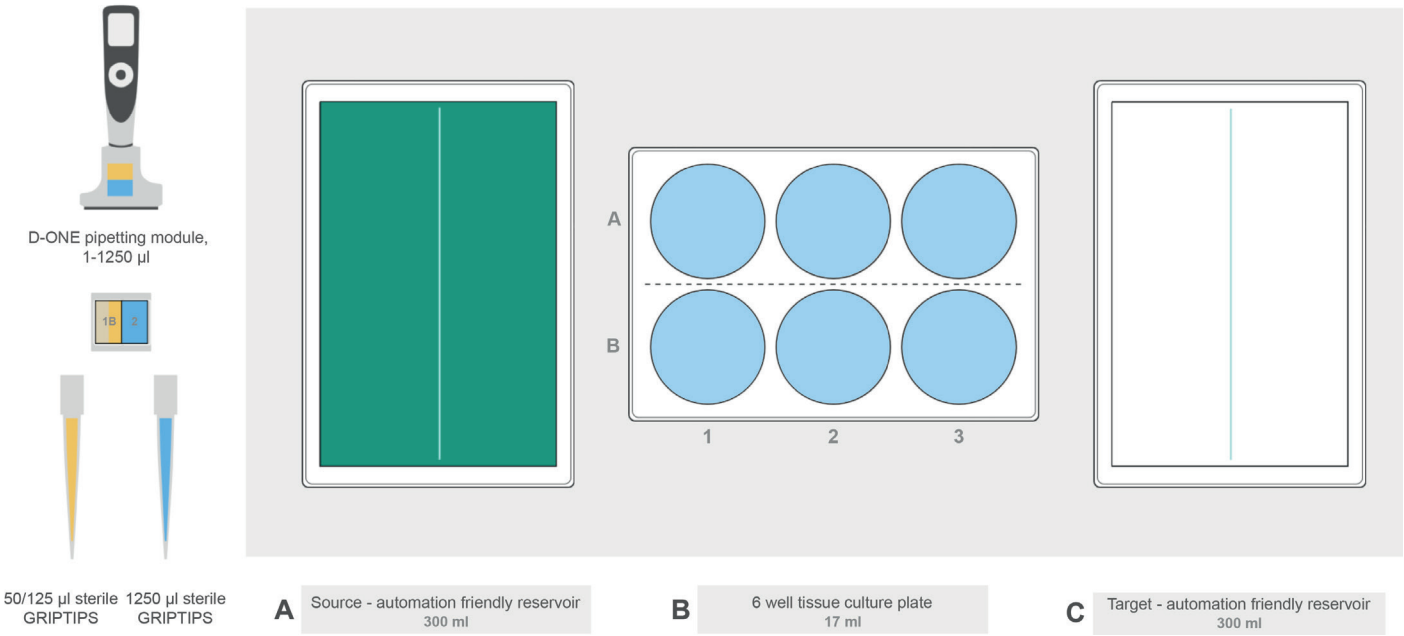

Figure 2: The ASSIST PLUS set-up for Bio-Blocks media exchange. **Position A:** a 300 ml automation friendly reservoir containing fresh media (green). **Position B:** 6-well plate with cells grown on Bio-Blocks (blue). **Position C:** an empty 300 ml automation friendly reservoir for media collection.

Step 2: **Media aliquoting for downstream processes**

How to: Following the completion of the media exchange, the ASSIST PLUS stops running and the pipette screen displays a message guiding the operator through the labware exchange. The operator should place a reservoir with the collected medium on deck position A and a tube rack holding 6 15 ml conical centrifuge tubes on deck C. The operator presses RUN to resume the program only after the labware change has been manually performed. 5 ml of collected media is then added to each centrifuge tube. If the medium needs to be processed separately for different cell types, tap the arrow in the center of the ASSIST PLUS display after the first medium transfer to pause the run. Exchange the 25 ml reservoir with a fresh insert, add the medium for the next well, then continue the program by pressing the arrow in the center of the ASSIST PLUS display again to resume the program.

Tips:

- For transfer volumes that exceed the pipette's maximum volume, a single transfer step with a multiplier is offered, allowing simpler programming
- Consider a labware change step in your VIALAB program when additional labware is needed on the deck of the ASSIST PLUS

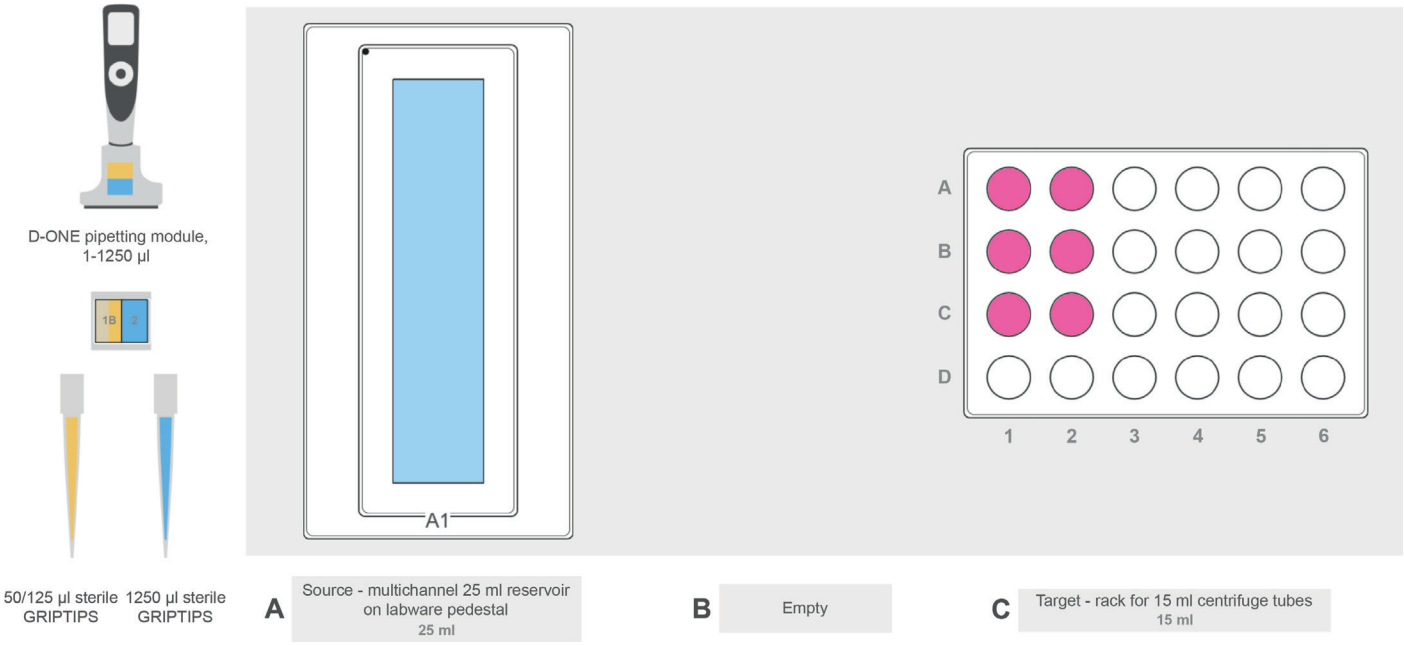

Figure 3: ASSIST PLUS labware layout 2 for aliquoting of preserved media. **Position A:** 25 ml multichannel reservoir with cell culture media collected from previous steps (blue). **Position B:** rack with 15 ml centrifuge tubes for distributing the collected media (pink).

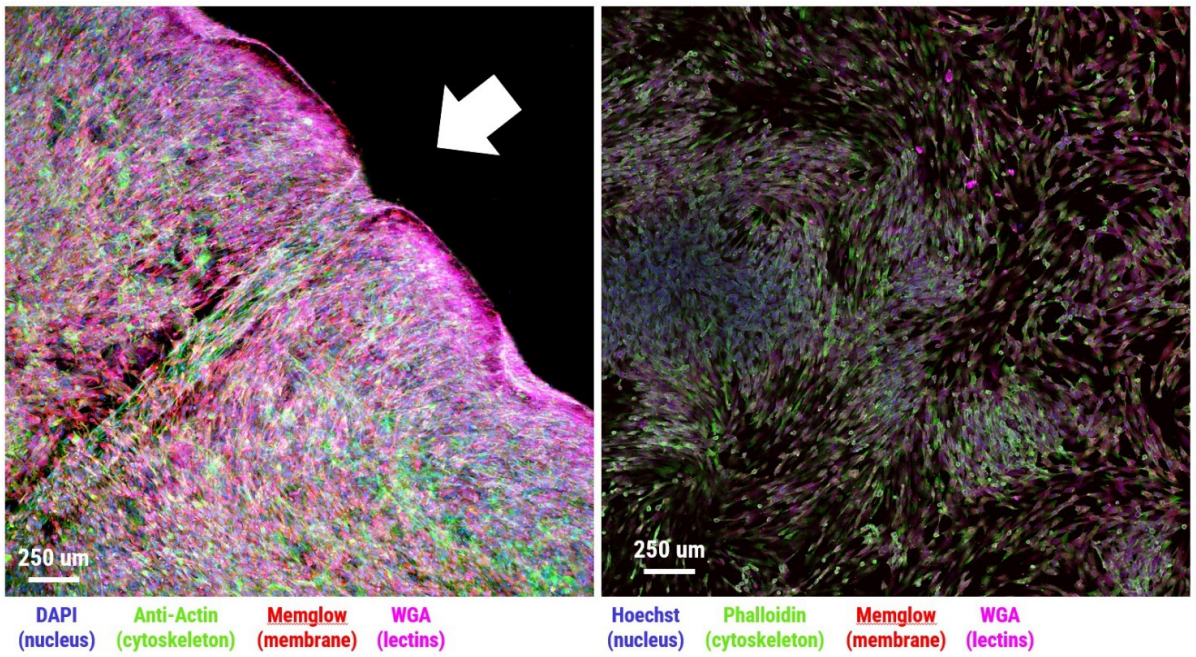

Figure 4: Adipose-derived stem cells (ASCs) cultured in 3D Bio-Blocks versus 2D monolayers. ASCs were grown at 37 °C at 5 % CO_2 in either Bio-Blocks or 2D flask for 2 weeks. (Left) In 3D Bio-Blocks, ASCs stained with DAPI (blue, nuclei), anti-actin (green, cytoskeleton), MemGlow™ (red, cell membrane) and WGA lectin (magenta, wheat germ agglutinin/extracellular matrix) exhibited dense cellular organization and enhanced ECM accumulation, particularly at the interface where 2 Bio-Blocks connected, indicating strong cell-matrix remodeling and inter-block integration (white arrow). (Right) ASCs in 2D monolayer cultures stained with Hoechst (blue, nuclei), phalloidin (green, cytoskeleton), MemGlow (red, membrane) and WGA lectin (magenta, ECM) showed planar alignment, fewer cells and limited ECM deposition. The Bio-Blocks system allows continuous ASC expansion without the need for enzymatic subculturing, as new cell-free Bio-Blocks can be added to promote further growth and matrix development. Scale bar = 250 µm. hydrogel; bar = 50 microns.ml multichannel reservoir (blue) and distributed to empty 15 ml centrifuge tubes (pink).

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Conclusion

- The integration of Ronawk's Bio-Blocks with the ASSIST PLUS robot and D-ONE single-channel pipetting module revolutionizes 3D biomanufacturing workflows. This approach optimizes reagent use, reduces media waste and requires a smaller laboratory footprint, translating to lower operational costs and increased throughput. This makes it a scalable, efficient and cost-effective solution for advanced 3D cell culture and EV biomanufacturing.
- Combining these technologies enhances viability, scalability and reproducibility, as well as reducing hands-on time for medium exchange and EV collection.
- The synergy between precise automation and physiologically relevant culture conditions accelerates breakthroughs and supports efficient high throughput applications, whether applied in regenerative medicine or other research fields.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS pipetting robot	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4532	D-ONE 5-1,250 µl Single Channel Pipetting Module	https://www.integra-biosciences.com/en/pipetting-robots/d-one-for-assist-plus
INTEGRA Biosciences	4535	D-ONE tip deck	https://www.integra-biosciences.com/en/pipetting-robots/d-one-for-assist-plus
INTEGRA Biosciences	4551	Labware Pedestal	https://www.integra-biosciences.com/en/pipetting-robots/d-one-for-assist-plus
INTEGRA Biosciences	6444	1,250 µl Sterile GRIPTIPS	https://www.integra-biosciences.com/en/griptips/automation-griptips
INTEGRA Biosciences	6327	300 ml Automation Friendly Reservoirs, polystyrene, sterile	https://www.integra-biosciences.com/en/reagent-reservoirs/automation-friendly-reagent-reservoirs
INTEGRA Biosciences	4310	25 ml Multichannel Reservoir	https://www.integra-biosciences.com/en/reagent-reservoirs/multichannel-reagent-reservoirs
INTEGRA Biosciences	4542	Tube rack for 15 ml conical tubes	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
CellVis	P06-1.5H-n	6 Well Culture Plate	https://www.cellvis.com/
Ronawk		Bio-Blocks	https://ronawk.com/

Contact us: