

# Wide Bore Orifice Automation Tips

## Comparison of standard vs. wide bore orifice tips using cell viability assay method

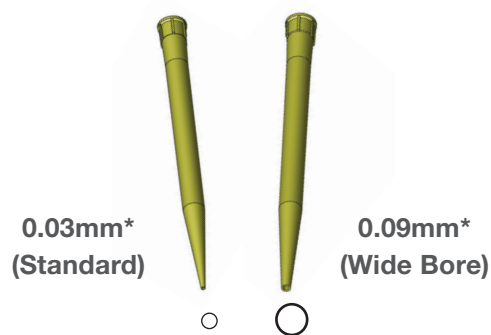
### Abstract

Due to the relatively small internal orifice diameter, using standard pipette tips may result in degraded biological sample integrity when pipetting fragile cell suspensions, hybridomas, protein aggregates and genomic materials. Thermo Scientific™ wide bore orifice tips are designed to address sample preparation challenges inherent in cellular, protein, and genomic material based samples by having a pipette tip orifice diameter that is three times the size of a standard pipette tip orifice. This application note illustrates a cell viability assay using the Invitrogen™ alamarBlue™ protocol on the Tecan™ Freedom EVO™ workstation; demonstrating how the output of viable cells increases when using Thermo Scientific wide bore orifice automation tips compared to standard automation tips.

### Introduction

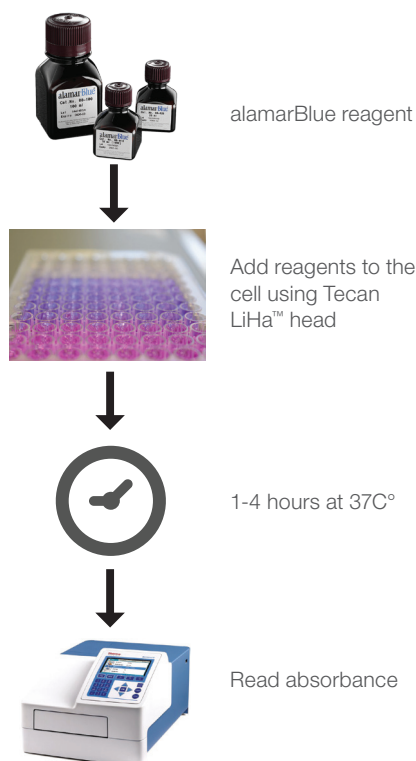
Cell viability assays are used in a wide range of applications. They can determine the health of a cell population and assist researchers in assessing the impact of environmental insult or drug toxicity on cell proliferation. Important performance characteristics of cell viability assays include accuracy, reproducibility, and speed. Wide bore orifice tips are designed to ensure biological sample integrity, reduce aggregate disruptions, and prevent tip clogging. Wide bore orifice tips coupled with automation can also provide highly accurate and precise well-to-well dispensing, yielding an excellent cells per well count.

### Comparison of Pipette Tip Orifice Diameters: Standard vs. Wide Bore



\*Enlarged for illustration purposes only

alamarBlue cell viability reagents function as a cell health indicator by using the reducing power of living cells to quantitatively measure the proliferation of various cell lines, including human, animal, bacteria, plant and fungi. This enables the user to establish relative cytotoxicity of agents within various chemical classes. When cells are alive, they maintain a consistent reduction within the cytosol of the cell. Resazurin, the active ingredient of alamarBlue, is a non-toxic, cell permeable compound that is a non-fluorescent blue color. Upon entering cells, resazurin is reduced to resorufin, a compound that is red in color and highly fluorescent. Viable cells continuously convert resazurin to resorufin, increasing the overall absorbance and color of the media surrounding the cells.



**Fig 1:** alamarBlue cell viability assay protocol. alamarBlue reagent is added directly to each well, the plates are incubated at 37°C to allow cells to convert resazurin to resorufin, and the absorbance signal is measured. Results are evaluated by plotting the absorbance signal versus cells/well.

The Tecan™ Freedom EVO™ workstation offers an advanced, proven and reliable liquid handling system for different scales and throughputs. This workstation provides an extendable work area and variable configurations as well as enabling the customer to automate a diverse range of applications including primary and secondary screening, cell growth and cell viability assays. Automation of cell viability assays on the Tecan Freedom EVO ensures accurate and precise dispensing of the reagent to a 96 well plate.

## Materials

1. Thermo Scientific Automation tips for Tecan Freedom EVO, standard and wide bore orifice:
  - a. P/N: 901-011 (standard)
  - b. P/N: 901-011G (wide bore orifice)
2. Tecan Freedom EVO LiHa head was used for all pipetting operations.
3. Invitrogen alamarBlue Cell Viability Reagent; Catalog no. DAL1100
4. Thermo Scientific™ Nunc™ MicroWell™ 96-Well Microplates; Catalog No. 269260
5. Thermo Scientific™ Multiskan™ FC Microplate Photometer

6. Thermo Scientific Series 800 Direct Heat CO<sub>2</sub> Incubator

7. Bacterial cells in tris buffer

## Method

Cell viability assay protocol was performed on the Tecan Freedom EVO workstations to compare yield between the standard and wide bore orifice tips.

1. Thaw bacterial cells and alamarBlue reagents and equilibrate to room temperature. Place cells and reagents in a deep well reservoir and 96 well assay plate, respectively, on the Tecan Freedom EVO workstation.
2. Perform a serial dilution using standard tips with the Tecan LiHa head, add 100 µl of bacterial cell plus the media to each well of a 96 well microplate.
3. Add 10 µl of alamarBlue reagent solution to the first well and pipette to mix. This well has the maximum number of cells.
4. Aspirate 100 µl from the first well and dispense into the next well, mix, and continue until the last well.
5. Aspirate 100 µl from the last well, which contains the minimum number of cells, and discard.
6. Repeat step 2 to 6 using the wide bore orifice tip.

Format	Volume of cells + medium	Volume of 10X alamarBlue to add	Tip type
96 well plate	100µl	10µl	Standard tip
96 well plate	100µl	10µl	Wide bore orifice tip

7. Incubate both assay plates at 37°C in a Thermo Scientific™ Series 800 Direct-Heat CO<sub>2</sub> Incubator for 4 hours.

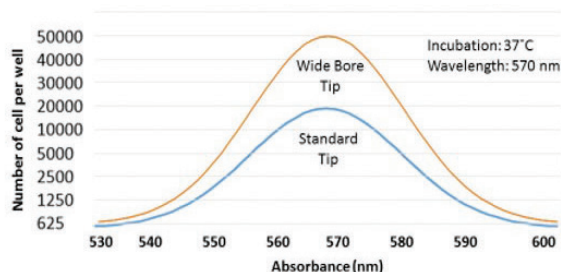
8. Transfer both plates to the plate reader and record the results by plotting absorbance versus compound concentration.

## Results

Serial dilution of resazurin in a cell culture medium was used to mimic the presence of the resorufin, a compound that is red in color and highly fluorescent. The serially

diluted bacteria cells demonstrated a bell curve response when the alamarBlue reagent was added. Absorbance was measured after 4 hours on the range of 530nm to 600nm on the Multiskan FC Microplate Photometer. The results demonstrates that the wide bore orifice tips have higher cells per well across the 96 well plate, indicating that cell lysis due to pipetting is significantly diminished by using wide bore orifice tips.

## alamarBlue Reagent Absorbance Standard Tip vs. Wide Bore Tip



**Fig 2:** The cells were incubated with alamarBlue reagent for 4 hours at 37°C, absorbance was measured at 570 nm.

## Conclusion

Choosing the right automation tip is important for generating high-quality data that answer your research questions with the greatest accuracy, speed and efficiency. This application note shows that by increasing the orifice diameter of a tip the cell count improves, ensuring the biological integrity of the samples.

Thermo Scientific wide bore orifice tips offer additional benefits not discussed in this application note:

- Reduce sample preparation time by allowing faster pipetting speeds of viscous reagent solutions, such as glycerol stocks or bead mixtures.
- Minimize tip clogging when pipetting fibrous sample matrices such as those found in genomic studies.

## Ordering Information

### Automation tips made to fit the Tecan and Beckman Automated Liquid Handling Systems

Product Code	Description	Packaging
903-011G	Tecan 175µl Conductive Sterile ART Barrier Genomic	96 Tips/Tray, 10 Trays/Pack
903-251G	Tecan 200µl Conductive Sterile Genomic	96 Tips/Tray, 10 Trays/Pack
903-252G	Tecan 200µl Conductive Non Sterile Genomic	96 Tips/Tray, 10 Trays/Pack
904-011G	Tecan 1000µl Conductive Sterile ART Barrier Genomic	96 Tips/Tray, 10 Trays/Pack
904-251G	Tecan 1000µl Conductive Sterile Genomic	96 Tips/Tray, 10 Trays/Pack
904-252G	Tecan 1000µl Conductive Non Sterile Genomic	96 Tips/Tray, 10 Trays/Pack
919-021G	Beckman FX 130µl Clear Sterile ART Barrier Genomic	96 Tips/Rack, 10 Racks/Pack, 5 Packs/Case
919-261G	Beckman FX 250µl Clear Sterile Genomic	96 Tips/Rack, 10 Racks/Pack, 5 Packs/Case
919-262G	Beckman FX 250µl Clear Non Sterile Genomic	96 Tips/Rack, 10 Racks/Pack, 5 Packs/Case

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