Assess the cell viability of *Staphylococcus aureus* biofilms

PrestoBlue HS and alamarBlue HS reagents for microplate viability assays.

Biofilms, a community life form of bacteria, are organized ensembles of sessile bacterial cells embedded in a self-produced matrix. The majority of chronic, antibiotic-resistant microbial infections are thought to be associated with biofilms. A signature trait of biofilms is their increased tolerance to antibiotics and to the host immune system, making successful eradication of biofilms a very challenging task. Different methods have been used for high-throughput screening (HTS) of biofilm inhibitors, primarily focused on measuring bacterial viability (i.e., metabolism) [1] or biofilm biomass [2]. Because biomass quantitation is typically a slow process and does not distinguish between live and dead cells, a more common first-tier approach to identifying biofilm inhibitors is to screen for the effects of investigational compounds on biofilm viability.

For HTS applications, a viability assay is ideally performed in an add-and-read format, requiring only three steps—addition of reagent, incubation, and measurement of output—with no mixing, washing, or cell lysis required. Add-and-read resazurin-based assays are commonly used to quantify metabolic activity of cells, including microorganisms. Upon entering live cells, resazurin is reduced to resorufin, a compound that is red in color and highly fluorescent. In addition to ease of use, resazurin-based assays provide extended linear ranges, ample signal windows, and the flexibility to measure in either endpoint or kinetic mode and with either fluorescence- or absorption-based instrumentation. The endpoint mode is often used in primary screenings, in which the goal is to perform fast screening campaigns to identify hit compounds from large chemical collections. The kinetic mode is more applicable to secondary screenings, in which the time-dependent cytotoxic effects of selected hits are assessed for mechanistic purposes. Resazurin-based assays have been extensively used in anti-biofilm screening approaches [3,4].

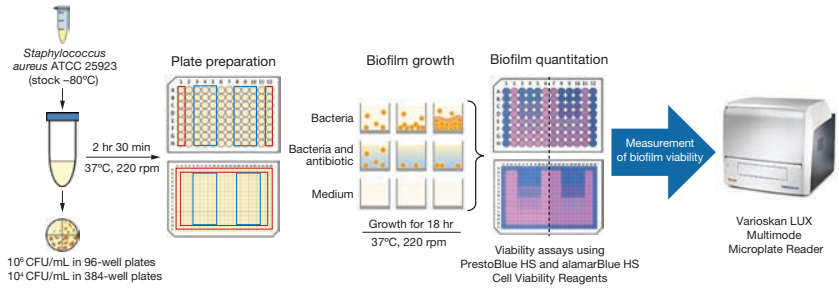

Figure 1. Workflow for quantitation of *S. aureus* biofilms with PrestoBlue HS and alamarBlue HS reagents. Exponentially grown cultures of *S. aureus* were prepared in tryptic soy broth medium as in [5] and added to microplates; medium-only controls were included. Biofilms were allowed to form without and with antibiotics at 37°C, 220 rpm; final assay volume was 200 µL in 96-well plates or 40 µL in 384-well plates. After 18 hr, planktonic cells were carefully removed, and biofilms were washed once with PBS prior to assaying with Invitrogen™ PrestoBlue™ HS or alamarBlue™ HS Cell Viability Reagent (Cat. No. P50201 or A50101). Resorufin fluorescence was measured using the Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader in either endpoint or kinetic mode.

PrestoBlue HS and alamarBlue HS reagents for anti-biofilm discovery

Two of the most popular resazurin-based dyes for cytotoxicity studies are the Invitrogen™ PrestoBlue™ and alamarBlue™ Cell Viability Reagents. Recently, Thermo Fisher Scientific introduced two new versions of these dyes—the Invitrogen™ PrestoBlue™ High Sensitivity (HS) and alamarBlue™ High Sensitivity (HS) Cell Viability Reagents—which contain highly purified resazurin (see "Just Released" on page 6). We have tested the suitability of PrestoBlue HS and alamarBlue HS reagents for measuring the viability of *Staphylococcus aureus* biofilms in an HTS workflow, such as that used when screening biofilm inhibitors. We have simulated the exposure to chemical libraries by performing a pilot study with model antibiotics, which were added to microplates just after the addition of *S. aureus* suspensions (Figure 1). The viability of attached biofilms was quantified in parallel using PrestoBlue HS and alamarBlue HS reagents. Fluorescence measurements of resorufin were performed using the Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader paired with Thermo Scientific™ SkanIt™ Software to allow fast readout of fluorescence signals and straightforward access to data processing steps such as calculations of cell viability, inhibition percentages of cell viability, and signal-to-background ratios.

Bacterial biofilm viability measurements: Endpoint mode

To model a primary screening workflow, PrestoBlue HS and alamarBlue HS reagents were used to assess biofilm viability with endpoint measurements. The reagents were added to *S. aureus* biofilms grown in 96- or 384-well plates and incubated at room temperature for

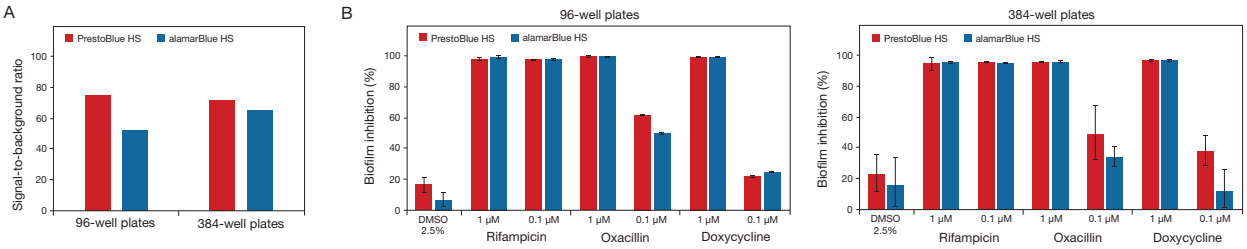

Figure 2. Performance of PrestoBlue HS and alamarBlue HS reagents in endpoint measurements of *S. aureus* biofilm viability. (A) Signal-to-background ratios were calculated from the fluorescent signals generated in untreated biofilm (signal) and media controls (background). **(B)** Rifampicin, oxacillin, and doxycycline were dissolved in anhydrous DMSO and added with the planktonic bacteria. After 18 hr, the quantity of formed biofilm per area was similar in the 96- and 384-well plates, as confirmed by viable plate counts (~1 x 10^8 CFU/cm^2). The viability assays with Invitrogen™ PrestoBlue™ HS and alamarBlue™ HS Cell Viability Reagents were performed as described in Figure 1. Fluorescence of resorufin was measured with top optics (12 nm excitation bandwidth) and excitation/emission = 560/590 nm using the Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader. All data were measured in two independent experiments, with 16 replicates (in A) and 2 replicates (in B) in each experiment.

40 minutes prior to fluorescence measurements with the Varioskan LUX reader (Figure 2A). The signal-to-background ratio was 2 to 3 times higher than historical values obtained with other commercial resazurin reagents [6,7], which can be attributed to a significant decrease in background fluorescence. Inhibitory effects of three model antibiotics were easily quantified, with rifampicin being the most active, consistent with reported findings [5]. For each antibiotic treatment, cell viability measurements using PrestoBlue HS reagent were noticeably similar to those using alamarBlue HS reagent in both 96- and 384-well plates (Figure 2B). These results demonstrate the suitability of both reagents for fast and reliable measurements of biofilm viability.

Bacterial biofilm viability measurements: Kinetic mode

In kinetic mode on the Varioskan LUX reader, biofilms incubated with either PrestoBlue HS or alamarBlue HS reagent showed linear increases in the production of resorufin during the 40-minute incubation. Both reagents exhibited kinetic rates that were remarkably similar, regardless of the biofilm's age (Figure 3), indicating that both reagents likely diffuse at similar rates through the matrix and are similarly metabolized. The inhibitory effects of antibiotics were also readily measurable in kinetic mode with both reagents. Killing of biofilms by doxycycline was less efficient once biofilms matured (Figure 3A vs. 3B), demonstrating the well-known resilience of biofilms to antimicrobial therapy.

Learn more about microplate assays for viability

Find out more about PrestoBlue HS and alamarBlue HS Cell Viability Reagents at **thermofisher.com/microplate-viability**. ■

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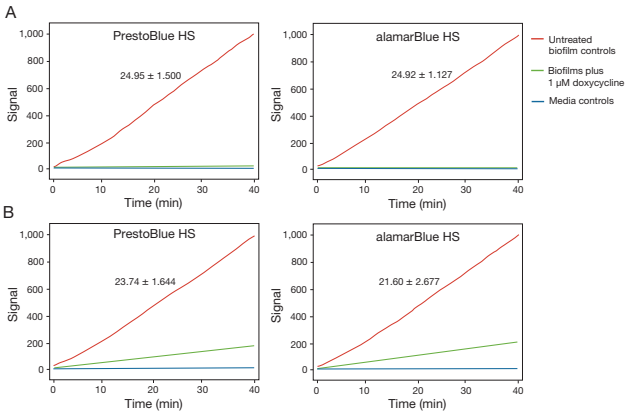

Figure 3. Kinetic curves of PrestoBlue HS and alamarBlue HS reagents showing linear resazurin reduction by *S. aureus* biofilms. Biofilms in 96-well plates were grown **(A)** for 18 hr in the presence of antibiotics or **(B)** for 18 hr and then an additional 24 hr in the presence of antibiotics; untreated biofilms and media controls were included. Invitrogen™ PrestoBlue™ HS or alamarBlue™ HS Cell Viability Reagent was added, and fluorescence was measured at excitation/emission = 560/590 nm every 2 min over 40 min using the Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader. The average fluorescence/min (indicated above red lines) was calculated for untreated biofilm controls using Thermo Scientific™ SkanIt™ Software.

References

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Product	Quantity	Cat. No.
alamarBlue™ HS Cell Viability Reagent	25 mL 100 mL	A50100 A50101
PrestoBlue™ HS Cell Viability Reagent	25 mL 100 mL	P50200 P50201
SkanIt™ Software for Microplate Readers, Research Edition	1 each	5187139
Varioskan™ LUX Multimode Microplate Reader	1 each	VL0000D0