# Assess cell health and function in 2D and 3D cell cultures

## Optimizing microplate assay protocols for spheroid cultures.

3D cell models are now increasingly being adopted for cancer, immuno-oncology, neuroscience, and other research areas because their microenvironments more closely resemble the micro-anatomy of *in vivo* systems (organized tissues, organs, and tumors), which contain a complex and dynamic set of cell types, chemical gradients, and extracellular matrix (ECM) components. The complexity of 3D cell structures can present a challenge when using cell health assays and protocols originally developed for monolayer (2D) cell cultures. Here we highlight a few basic considerations for adapting and optimizing our microplate-based 2D cell culture viability assays for spheroid cultures, focusing on the optimization of reagent concentration and incubation time.

### Determine the optimal reagent concentration

The optimal reagent concentration for spheroid cultures will provide a high assay-specific signal with the lowest possible nonspecific background, resulting in a high signal-to-noise (S/N) ratio. An assay with a high S/N ratio shows greater sensitivity for detecting changes in cell health due to cell treatments, such as exposure to drugs or test compounds. To demonstrate this effect, we assayed spheroids derived from human lung epithelial cells (A549 cells) using the Invitrogen™ CyQUANT™ XTT Cell Viability Assay, a colorimetric microplate assay originally developed to assess 2D cell viability as a function of redox potential (Figure 1). We compared the recommended reagent concentration (1X) with a 2X concentration, in the absence or presence of increasing concentrations of the cytotoxic drug gambogic acid, and found that doubling the reagent concentration increased the S/N ratio, and thus the assay sensitivity. We recommend testing a wide range of reagent concentrations to find the most appropriate one for your 3D assay.

### Determine the optimal reagent incubation time

The optimal reagent incubation time for spheroid cultures can be determined by running a time-course experiment and plotting assay signal as a function of incubation time. Figure 2 shows an example of such a time-course experiment using an established microplate viability assay that determines redox potential by measuring the reduction of resazurin to the highly fluorescent (and intensely colored) resorufin. Using the Invitrogen™ PrestoBlue™ HS Cell Viability Reagent with various incubation times, we compared the fluorescence generated by A549 cells in a 2D monolayer with that of A549 cell–derived spheroids. The assay protocol recommends incubation times for 2D cell culture of 10 minutes to 3 hours, within the linear range of the time-course curve. Based on our experiments with spheroids, we recommend extending the incubation time—to between 5 and 10 hours—to maximize signal and stay within the linear range of this curve.

### Learn more about microplate assays for 3D cultures

Thermo Fisher Scientific offers a wide range of microplate assays for the detection of cell viability, health, and function that can be easily optimized for 3D cell culture systems. Find out more about our microplate assays and microplate readers at **thermofisher.com/microplateassays**, and explore our 3D cell culture products at **thermofisher.com/spheroid**. ■

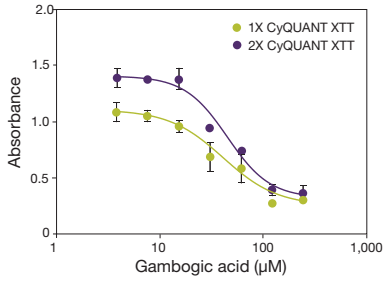


**Figure 1. Determine the optimal reagent concentration.** Human lung epithelial cells (A549 cells) were plated in Thermo Scientific™ Nunclon™ Sphera™ 96U-Well Microplates (Cat. No. 174925) at 5,000 cells/well in complete MEM for 19 hr to allow spheroid formation. The surface of these plates exhibits extremely low ECM binding properties, which inhibits cell attachment and promotes spheroid formation. The A549 cell spheroids were then treated with 7 concentrations of gambogic acid for 26 hr and assayed for cell health with the Invitrogen™ CyQUANT™ XTT Cell Viability Assay (Cat. No. X12223) using the recommended (1X) or 2X reagent concentration. All measurements were made using the Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader (Cat. No. VLBLATD0).

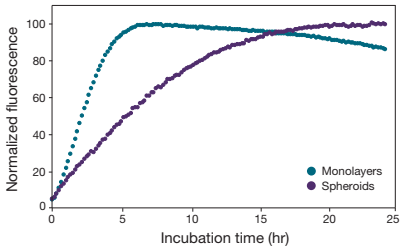


**Figure 2. Determine the optimal reagent incubation time.** A549 cell spheroids (prepared as described in Figure 1) and A549 cell monolayers were assayed at various incubation times with Invitrogen™ PrestoBlue™ HS Cell Viability Reagent (Cat. No. P50200). All measurements were made using the Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader (Cat. No. VLBLATD0).

| Product | Quantity | Cat. No. |
|---|---|---|
| CyQUANT™ XTT Cell Viability Assay | 1 kit | X12223 |
| PrestoBlue™ HS Cell Viability Reagent | 25 mL<br>100 mL | P50200<br>P50201 |
| Varioskan™ LUX Multimode Microplate Reader | 1 each | VLBLATD0 |