BIOGEN Científica
COMPROMETIDOS CON LA INVESTIGACIÓN DESDE 1989

Cell-based assays on microplate readers

Cellular microplate assays are popular tools in life science. They enable studying cellular events that cannot be analyzed in isolated cell components. Monitoring such cellular processes over time expands the output of an experiment as it allows comparing time and extent of a biological reaction and as it prevents to miss the time point of reaction. Here, the microplate format is advantageous since various conditions can be analyzed in parallel.

Recording cell-based assays in microplates requires specific characteristics of assays as well as of the measurement equipment. Obviously, a suitable microplate reader needs to be able to read the detection mode determined by the assay, for cellular assays these are predominantly fluorescence intensity, luminescence, absorbance, time-resolved fluorescence or combinations thereof. The opportunity to measure from either top or bottom of the microplate is desired to measure adherent cells and may circumvent disturbances of the measurement by cell culture medium components or condensation. An absolute requirement is the possibility to control atmosphere and temperature within the measurement chamber of the microplate reader. In order to keep human cell lines viable for the duration of the experiment, they need to be incubated at 37 °C and at a CO_2 concentration of 5 % to maintain the physiologic pH provided by a bicarbonate buffer. Since the oxygen available in human tissues is typically below ambient, it is further recommended to run real-time cellular assays at defined oxygen conditions and thereby mimic physiologic conditions.
Furthermore, assay components must not be cytotoxic and should not interfere with the analyzed process itself. The method must be stable for the time of analysis and at temperatures of 37 °C, the temperature human and other mammalian cells are mostly cultured at. If the cellular reaction is reported by a change in fluorescence, the fluorophore needs to be resistant to bleaching and ideally emits light rather in the red range of the visible spectrum in order to be distinguishable from cellular autofluorescence.

Nowadays, there is a huge range of cell-based assays available which was optimized for microplate measurement. The following two applications serve as examples of methods which are widely employed as internal controls in cell-based research.

Determination of transfection efficiency

Transfection experiments are frequently used in cell-based studies. Typically, the success of a transfection is determined microscopically using fluorescent reporter proteins. However, microplate readers are equally suited for this purpose and offer further advantages such as the quantitative, fast, and automatic determination of transfection efficiency.

For this purpose, HeLa cells were transfected with the genetic sequences for GFP and mcherry and mixed in increasing ratios with WT-HeLa cells without fluorescent reporter and read on a fluorescence microplate reader. The results in fig. 1 and 2 confirm a linear relationship between the percentage of GFP+/mcherry+ HeLas (= transfection efficiency) and the measured signal for GFP or mcherry fluorescence with high accuracy (R^2 = 0,9997 and 0,9998) and precision (%CV = 10.5 and 5.2) while Hoechst-staining was used as reference for total cell counts. This experimental approach allowed to reliably detect cells expressing a fluorescent marker down to ~600 cells/well in a 96-well plate.

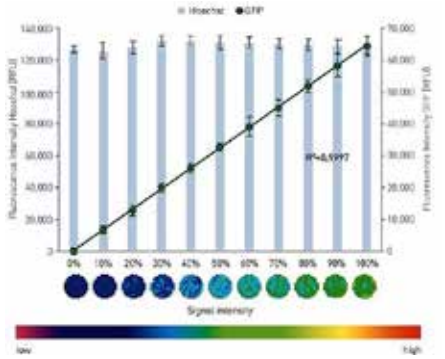


Fig. 1: Linear relationship of percentage of GFP+/mcherry+-HeLas (=transfection efficiency) and obtained GFP signal with matrix scan. Error bars refer to 8 replicates. Matrix scan example shown for one well each.

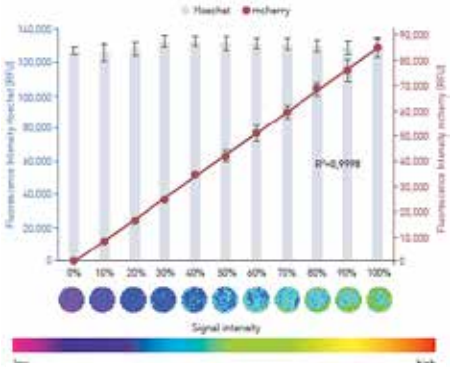


Fig. 2: Linear relationship of percentage of GFP+/mcherry+-HeLas (= transfection efficiency) and obtained mcherry signal with matrix scan. Error bars refer to 8 replicates. Matrix scan example shown for one well each.

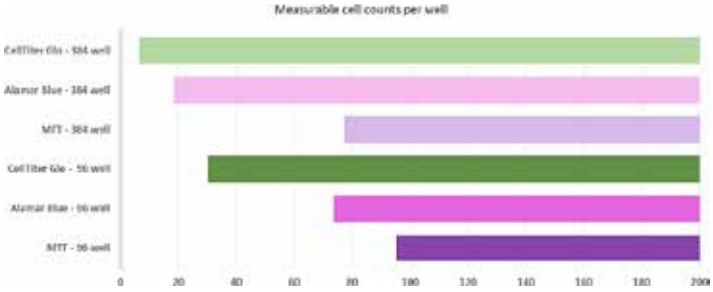
Cell viability determination

The investigation of cell viability is another application which is frequently employed in cell-based research. This flexible method can be used in several ways, for example to screen for cytotoxic compounds, crucial in cancer related research, or to evaluate the condition of a cell culture. Cell viability is determined by different means, most often by the detection of metabolic activity in a cell culture.

For this purpose, there is a huge range of commercially available kits to choose from which utilize different detection modes. Multimode microplate readers offer the ideal measurement platform for these kits and can be used for the detection of all commonly used viability assays.

In comparison, these assays offer different limits of detection (LOD) which refer to the lowest number of living cells detectable in a sample. However, as depicted in figure 3, the LOD not only depends on the assay, but can also be influenced by the used plate type. While the luminescence- and fluorescence-based methods CelllTiter Glo and Alamar Blue were better suited to detect lower cell counts in comparison to the absorbance-based MTT assay, best results for all three assays were achieved in 384-well plates.

Fig. 3: Comparison of viability assay kits in terms of their lower detection limit using HeLa cells.



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