

pH –Xtra Product Information

PRODUCT NAME

pH-Xtra

Assay Principle

pH-Xtra probe monitors cellular acid extrusion. Probe phosphorescence signal is modulated by pH such that increased acidification causes increased phosphorescence signal.

ASSAY FORMAT

Easy 'mix and measure' plate-based assay using standard 96 well plates.

PRODUCT DESCRIPTION

pH-Xtra (1vial/96-well plate) comprises of dried phosphorescent pH-sensitive probe used when reconstituted in aqueous solution. The probe is chemically stable and inert. It is cell impermeable and contains no special additives. It is excitable at 340-410 nm and has emission maxima at 590, 615 and 690 nm.

SAMPLE PREPARATION

To prepare a stock solution of the probe add 1 ml of water or assay buffer to the vial provided. Add 10 μ l of this solution to each test well.

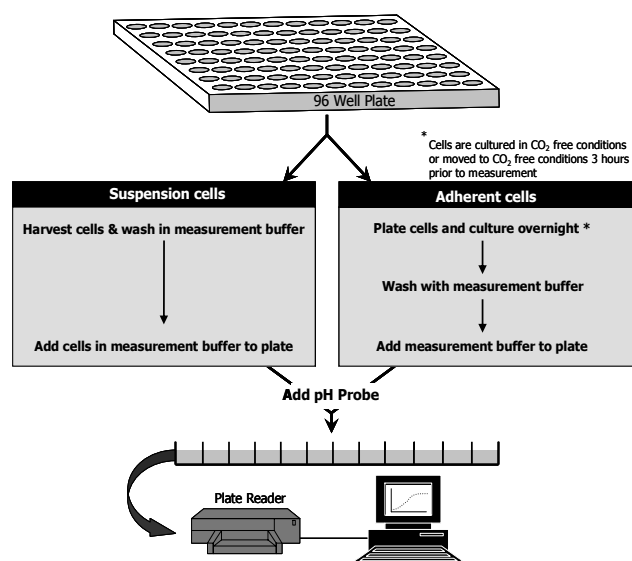
PHOSPHORESCENT MEASUREMENTS

The pH-Xtra probe is measured using dual delay time-resolved measurements (recommended reader for such measurement is the Victor, PerkinElmer). Optimal measurement wavelengths are 340 nm excitation and 615 nm emission. Delay times of 100 and 300 μ s used, both with a measurement window of 30 μ s respectively. These dual intensity measurements are used to calculate emission lifetime using the following function: $\tau = t_2 - t_1 / \ln(D_1/D_2)$ [t =delay time, D =measured intensity value].

The measurement solution should have a low buffering capacity. Options include commercially available unbuffered DMEM or 1mM PBS containing 20mM Glucose, 4.5g/l NaCl, 4g/l KCl, 0.097g/l MgSO₄, 0.265g/l CaCl₂. Warm media to 30°C, and pH to 7.4 prior to use.

EXAMPLE ASSAY PROCEEDURE

Prepare plates on a plate heater equilibrated to 30° C. Add cells to test wells (suspension/trypsinised adherent cells) prior to measurement. Alternatively adherent cells may be pre-plated. Cells may be cultured in CO₂ free conditions or moved to such conditions 3 hours prior to measurement. Reconstitute the pH-Xtra probe in 1 ml H₂O and dispense 10 μ l of this stock solution into to each well. For compound testing, add 1 μ l of compound in an appropriate solvent to test wells. For measurement, insert the prepared plate into a fluorescence plate reader pre-set to 30° C, measuring at 1.5 min intervals for ~40 min using the optical settings outlined above. Extracellular acidification is measured by assessing the rate of probe signal increase. For semi-quantitative analysis the rate of signal increase can be used to compare treated and untreated samples. For more quantitative analysis, these data may be converted to [H⁺] concentrations using a simple transformation. For further information see www.luxcel.com



Assay Schematic



pH-Xtra Materials Safety Data Sheet

PRODUCT NAME

pH-Xtra

CATALOGUE NUMBER

PH-100

UNIT SIZE

1 vial/96-well plate

MANUFACTURER

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COMPOSITION/INFORMATION ON INGREDIENTS

Phosphorescent pH-sensitive probe, water-soluble material.

HANDLING AND STORAGE

Recommended storage of dry material: store at +4°C.

Recommended storage of product reconstituted in aqueous: store at -20°C and avoid freeze/thaw cycles.

Protect product from prolonged illumination and exposure to light, may be exposed to light for short time-periods.

OTHER INFORMATION

This material is not considered as hazardous, it is not present above 1%, nor is it a carcinogen above 0.1% as defined in 29 CFR 1910.1200, the OSHA Hazard Communication Standard. Therefore, a Material Data Sheet is not required. We recommend treating all chemicals with caution.

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