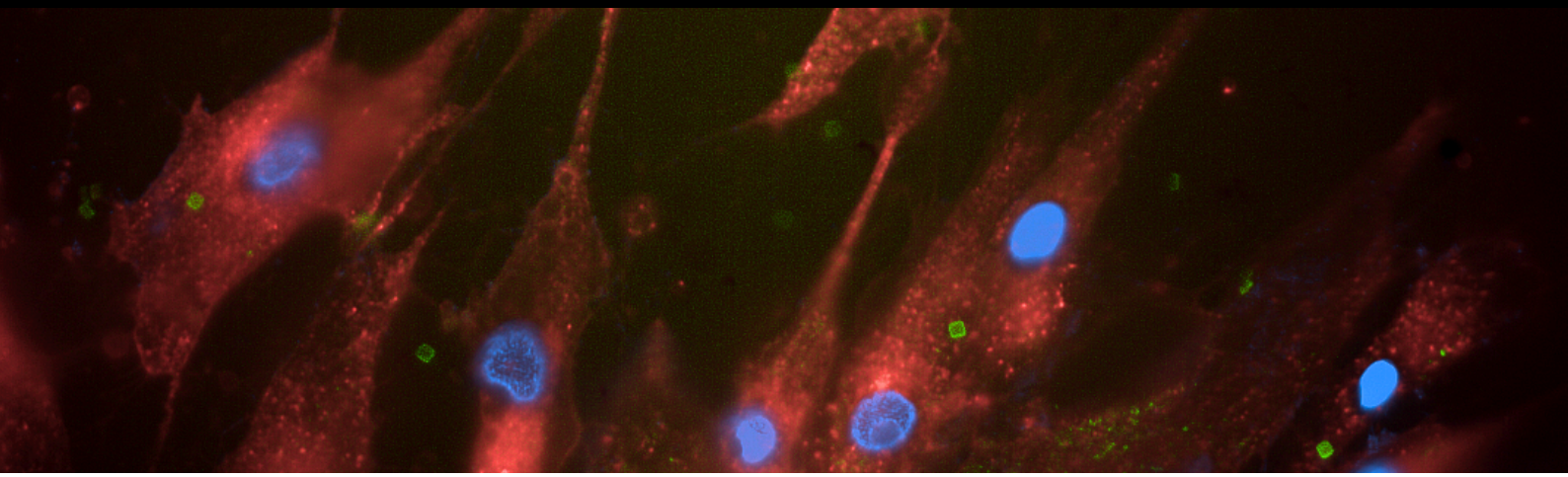
Real-time intracellular hydroxyl radical detection for ROS monitoring in live cells:

CytoCHECK SPAchip® **OHrad ROS** Single-Detection Kit NEW



Reactive oxygen species (ROS) term is commonly used to define the reactive molecules and free radicals originating from molecular oxygen. A large number of studies have proved the messenger role of ROS in cell survival, proliferation, differentiation, cell death, apoptosis, and ferroptosis (1,2).

CytoCHECK SPAchip® technology facilitates surface modification with a fluorescent dye, enabling real-time monitoring of intracellular ROS through population to single-cell analysis. **CytoCHECK SPAchip® OHrad ROS Single-Detection Kit** specifically enables the detection of **hydroxyl radicals (•OH)** via changes in green fluorescence intensity within living cells. In addition, this product has been designed to only be active when in contact with intracellular esterases.

Now, **CytoCHECK SPAchip® OHrad ROS Single-Detection Kit** offers an innovative solution: it can monitor **intracellular hydroxyl radical levels without being affected by pH changes of the media**; thereby enabling the dynamic detection inside living cells without any perturbation of their physiological or metabolic state over time.

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APPLICATIONS

01 NEURODEGENERATIVE DISEASES

How oxidative stress contributes to neurodegenerative diseases, including neuron damage and mitochondrial dysfunction. For example, Amyotrophic Lateral Sclerosis (ALS) studies on the role of hydroxyl radicals in the degeneration of motor neurons.

02 CANCER THERAPIES

How antitumoral drugs may induce hydroxyl radicals leading to apoptotic cell death and ferroptosis (1,2). Therapy assays using antioxidants to reduce oxidative stress in cancer cells to improve the effectiveness of traditional treatments such as chemotherapy and radiotherapy.

03 CARDIOVASCULAR DISEASES

How oxidative stress and hydroxyl radicals contribute to atherosclerotic plaque formation and endothelial dysfunction. Studies on oxidative damage induced by reperfusion of ischemic tissues.

04 DIABETES AND METABOLIC ACTIVITY

How excess glucose and metabolic disorders induce the generation of hydroxyl radicals, contributing to diabetes complications.

05 AGING RESEARCH

How oxidative stress contributes to cellular aging and the deterioration of biological functions with age. Research on the relationship between oxidative stress and diseases such as osteoporosis and macular degeneration.

06 INFLAMMATORY AND AUTOIMMUNE DISEASES

Studies on how ROS, including hydroxyl radicals, act as mediators of inflammation and contribue to pathogenesis of chronic inflammatory and autoimmune diseases.

Monitoring intracellular radical hydroxyl (•OH) for reactive oxygen species (ROS) detection in 1095SK fibroblast cell line

SPAchip® technology is optimal to work in the intracellular environment and has the ability to remain in the cytoplasm for long periods of time (3). **CytoCHECK SPAchip® OHrad ROS Kit** has been designed to be active ONLY when in contact with intracellular esterases, thus obtaining a fluorescent signal with internalized SPAchips.

KEY FEATURES:

- **ROS sensor:** intracellular activation by cytosolic esterases.
- **ROS specificity:** hydroxyl radical (•OH).
- **pH-insensitive.**

CytoCHECK SPAchip® OHrad ROS Single-Detection Kit is **selective for hydroxyl radicals (•OH)**. In cells, •OH radical can be generated spontaneously from H_2O_2 through the Fenton and Haber-Weiss reactions catalyzed by metal ions (4). H_2O_2 is formed by superoxide dismutase (SOD) from superoxide radical anion (O2•-). Hence, this kit enables to measure •OH radicals generated either as primary ROS or as secondary species downstream of other ROS.

Additionally, OHrad ROS SPAchips are **not sensitive to pH changes** (Fig. 1), avoiding measurement artifacts due to pH variations in experimental setup.

The use of **Doxorubicin (DOXO)**, an anthracycline that has been broadly used in the treatment of malignant tumors due to its broad spectrum, efficiency, and induction to oxidative stress, results in excessive reactive oxygen species (ROS) generation (5,6). DOXO produces ROS by redox cycling at its semiquinone structure (6,7). As reported in the Fenton's reaction, iron catalyses the generation of •OH radicals from H_2O_2 (7,8).

Here, we analyzed reactive oxygen (ROS) changes in **1095SK fibroblast cells** using **CytoCHECK SPAchip® OHrad ROS Single-Detection Kit**. Firstly, we observed internalized OHrad ROS SPAchips in green fluorescence channel in fibroblast cells (Fig. 2). Secondly, ROS concentration analysis in 1095SK cell line was performed, observing that SPAchip signal is dependent on the ROS amount produced by the cells due to drug concentration (3 and 6 hours after DOXO treatment) (Fig. 3). SPAchip fluorescence signals showed an increment in the intensity (compared with basal condition) as a function of the drug concentration (Fig. 3).

Thus, **CytoCHECK SPAchip® OHrad ROS Single-Detection Kit** allows to easily detect **intracellular hydroxyl radical dynamics inside live cells** (e.g. 1095SK fibroblast cells; Fig. 2) without causing cytotoxicity over long periods of time. In addition, our SPAchip biosensors facilitate accurately monitoring and measurement of reactive oxygen species **without being affected by the pH of media** (Fig. 1).

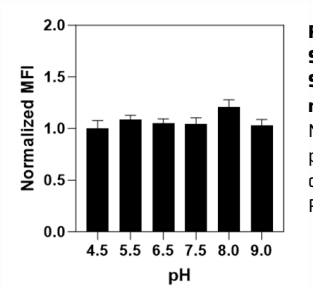

Figure 1: CytoCHECK SPAchip® OHrad ROS Single-Detection Kit is not pH-sensitive. No significant effect of pH on the fluorescence of CytoCHECK OHrad ROS SPAchips®.

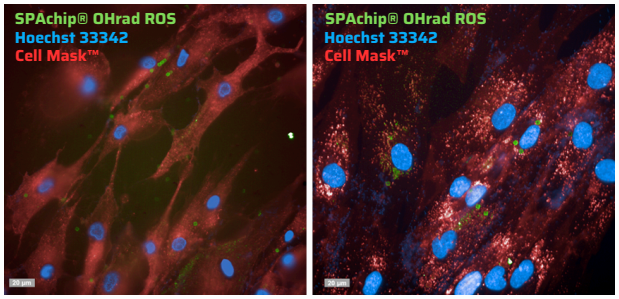

Figure 2: CytoCHECK SPAchip® OHrad ROS Single-Detection Kit in fibroblast 1095SK cell line. Images showing intracellular ROS SPAchip® in green, acquired with 40x magnification objective. In blue, nuclear staining by Hoechst 33342. In red, cytoplasmatic staining by Cell Mask™ Plasma Membrane. Scale bar: 20 µm.

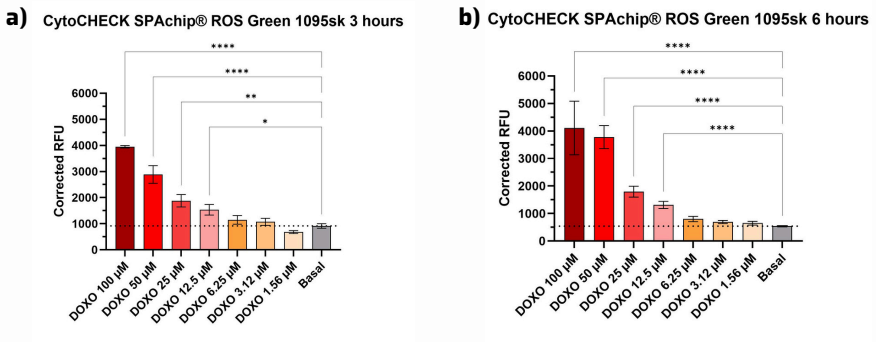

Figure 3: Doxorubicin dose-response with CytoCHECK SPAchip® OHrad ROS Single-Detection Kit in fibroblast 1095SK cell line. Fluorescence corrected intensity of ROS detection probe coupled to SPAchips within fibroblast 1095sk cell line after 3 hours **a)** and 6 hours **b)** of treatment with different Doxorubicin (DOXO) drug concentrations. ROS SPAchips exhibit a correlation with the treatment concentration. Bars represent mean values for each condition and error bars correspond to SEM values. ROUT method was used for identifying outliers. For statistical analysis, two-way ANOVA with alpha value set to 0.05 (95% confidence interval) was performed.

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