

## HIGH CONTENT CARDIOTOXICITY ASSAY

### CELL BASED ASSAYS

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<b>Service:</b>	High Content Cardiotoxicity Assay
<b>Available Host Cells:</b>	Human / Rat / Mouse Cardiomyocytes Human Cardiac Progenitor Cells
<b>Stimulation:</b>	Compounds to be tested

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#### **Background**

Cardiotoxicity is a condition when the heart has problems to pump properly. One of the most common events for a heart dysfunction is the toxicity of the functional cardiac cells – cardiomyocytes-. Cardiomyocytes are the muscle cells of the heart that induce the pumping of the organ. The toxicities that affect these cells compromise seriously the organ function of the affected organism. It is well known that several drugs have side effect in the cardiomyocytes and the capacity to detect this effect in chemical compounds is crucial in the drug discovery. The most usual mechanism involved in the cardiac cell dysfunction is the oxidative stress, the mitochondrial dysfunction and the caspase activation with the subsequent apoptosis.

#### **Outcome Parameters**

- Oxidative stress
- Mitochondrial Damage
- Apoptosis
- Cell Viability

#### **Compound testing**

In this assay we predict the cardiotoxicity of the compounds measuring the most important parameters affected by the drugs: Oxidative stress, mitochondrial damage and apoptosis. Human or animal primary cardiomyocytes are treated with the drugs at several concentrations and the results are compared with the positive controls of cardiotoxicants. We measure the mitochondrial damage with the CM-H2DCFDA probe. This probe measure mitochondrial membrane potential and the results are correlated with the mitochondrial integrity. The oxidative stress is measured quantifying the reactive oxygen strains using the TMRM. Meanwhile, we use an antibody against active caspase-3 protein to measure DNA damage. The cardiotoxic capacity of the compounds is measure quantifying these three parameters and correlating their values with positive controls like “doxorubicin” and “daunorubicin” (Anderson et. Al. 1996), two anticancer drugs that produce negative effects in cardiomyocytes.

**Sample Assay**

Human or animal cardiomyocytes are treated with the compounds during established incubation period (12 hours Daunorubicin, 24 hours Doxorubicin) at different concentrations and after the treatment two probes are added to measure the oxidative stress and mitochondrial damage. After the probes measurement, the immunohistochemistry for active caspase 3 is performed. Results from the different parameters are compared with negative controls for each parameter and the drug will be refused in the case that the differences for one parameter could be statistically significant in comparison with the negative controls. The experiment has been tested with the compounds that are used like references for the different parameters. "doxorubicin" and "daunorubicin" are drugs that affect the three parameters.

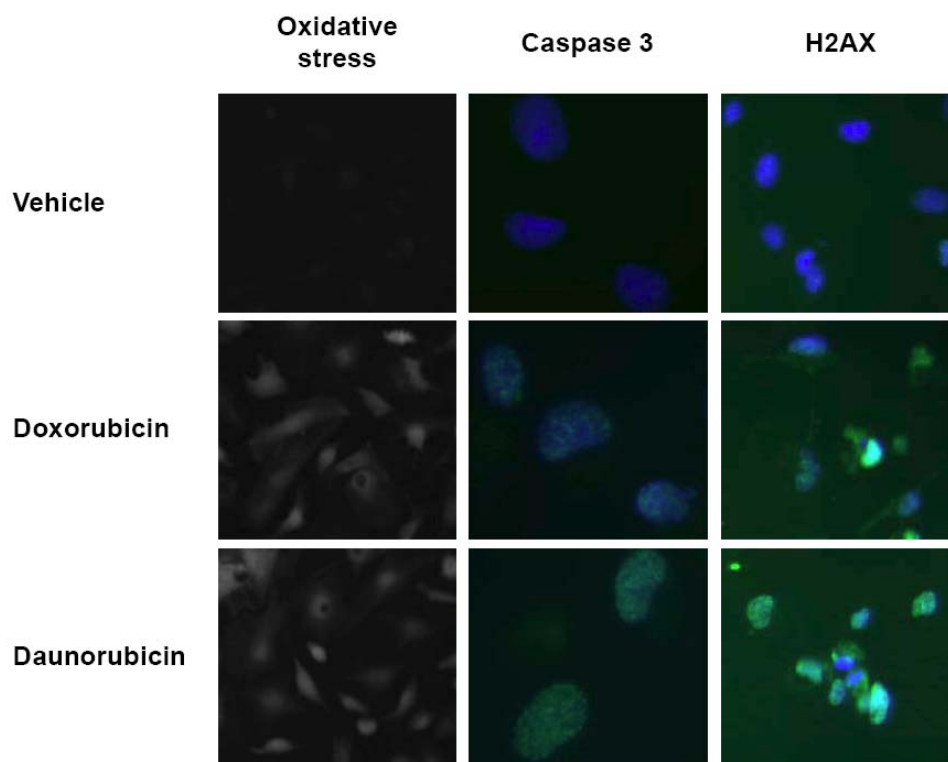


Figure 1. Representative images of the three analyzed parameters.

