

## **SOP: Cytotoxicity. The LDH Assay (JUMC)**

Reagents:)

**CytoTox 96® Non-Radioactive Cytotoxicity Assay** (Promega cat. No.G1780.

Kit contents:

- Substrate Mix
- Assay Buffer
- Stop Solution

### **Required equipment:**

fresh 96 well plate (Corning)

### **Preparation of working solution:**

1. Warm the 12ml of Assay Buffer to room temperature (keep protected from light).
2. Add the 12ml of room temperature Assay Buffer to a bottle of Substrate Mix. Invert and shake gently to dissolve the substrate. (Once resuspended, protect the Substrate from strong direct light and use immediately)

### **Procedure :**

1. Incubation of cells with investigated factors/substances for certain time according to experimental protocol..
2. Transfer 30µl supernatant (medium) to enzymatic assay plate.
3. Add used for culture medium to separate wells for background control.
4. Add 1:5,000 dilution of LDH Positive Control to an empty well without cells.
5. Add 30µl reconstituted Substrate Mix to each well of enzymatic assay plate.
6. Cover plate and incubate for 30 minutes at room temperature, protected from light.
7. Add 30µl Stop Solution to each well.
8. Record absorbance at 490nm.