

## Standard Operating Procedure @ CNR- IREA

PROTOCOL	Cell cultures exposure to UVB radiation
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### Index

<b>1. Purpose</b>	1
<b>2. Equipments and Materials</b>	1
<b>3. Procedure</b>	2
<b>3.1. UVB irradiance measurements</b>	2
<b>3.2. Preparation of cell samples</b>	3
<b>3.3. UVB exposure</b>	3

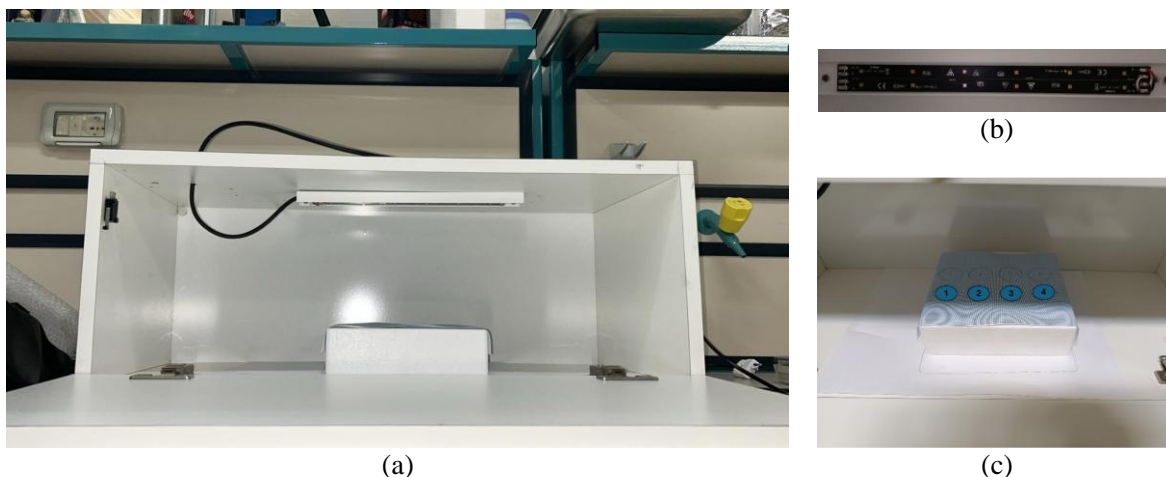
### 1. Purpose

This procedure describes the equipment and the protocol used for cell cultures exposure to UVB radiation. The aim is to ensure consistency and compliance of the experiments with good laboratory practices.

### 2. Equipments and Materials

- Customized wooden box (710 mm long, 260 mm wide, 300 mm deep)
- UVB LED board with six LEDs each (LITE-ON, part number LTPL-G35UV308GH, UVB LED 308 nm, 45 mW, 350 mA, 120°, ERI Lighting S.R.L)
- LED AC/DC power supply (Professionale DALI, TCI srl)
- Handheld radiometer (DeltaOhm, HD2102.1) and UVB (LP471UVB, 280 – 315 nm) probe

- Styrofoam holder 6 cm high
- 35 mm cell culture dish (Corning, cod. 430165)



**Figure 1. Exposure system setup.** (a) UVB exposure set-up; (b) LED board; (c) irradiance measurement positions

### 3. Procedure

#### 3.1. UVB irradiance measurements

- Wear personal protective equipment (gloves and glasses)
- Turn on the lamp at least 30 minutes before the start of measurement to allow warm-up
- Connect the UVB probe to the HD2102.1 (DeltaOhm) radiometer
- Switch-on the radiometer and wait until the completion of the auto-calibration procedure
- Turn the lamp off to allow placing the probe in the measurement position 1 according to Figure 1(c). STEP 1
- Close the wooden box and turn the lamp on. STEP 2
- Wait 2 min to assure stabilization of the UVB irradiance level. STEP 3
- Acquire the average, minimum and maximum UVB irradiance levels over 2 min time interval. STEP 4
- Repeat STEPS 1-4 for the remaining measurement positions (2, 3 and 4 in Figure 1(c))
- Repeat the whole procedure of UVB irradiance measurements at least three times for each position

- Periodically check the irradiance levels before proceeding with the UVB exposure of cell cultures

### 3.2. Preparation of cell samples

- Prepare the following samples from the same batch of cells: incubator control, negative control (cell culture kept on the bench outside the wooden box for the whole UVB exposure duration) , UVB exposed, positive control.
- Label the cell culture dishes to decode each sample upon completion of the analysis for blind experiments: the operator who performs the analysis is not aware of the sample in hand

### 3.3. UVB exposure

- Wear personal protective equipments (gloves and glasses)
- Turn the lamp on at least 30 minutes before the start of exposure to allow warm-up
- Replace the culture medium with 1 ml warmed PBS to avoid the interference of red phenol during the UV exposure
- Locate the dishes on the labelled positions of the styrofoam holder (Figure 1(c)) for UVB exposure and outside the box for negative control
- At the end of the exposure, turn off the lamp
- Open the box and carefully remove the culture dishes
- Proceed with the harvest and the procedure for the biological assay by following the related SOP