

## Standard Operating Procedure @ CNR- IREA

PROTOCOL	Cell cultures exposure to 5G signal at 26.5 GHz
DATE	01/12/2023
AUTHOR(S)	Mariateresa Allocca, Stefania Romeo, Anna Sannino, Maria Rosaria Scarfi, Olga Zeni
REVISED BY	Maria Rosaria Scarfi, Olga Zeni
APPROVED BY	Olga Zeni

### Index

1. Purpose.....	1
2. Equipments and Materials.....	1
3. Procedure .....	2
3.1. Preparation of cell samples.....	2
3.2. RF exposure .....	3

## 1. Purpose

This procedure describes the equipment and the protocol used for cell cultures exposure to electromagnetic fields (EMF) at 26.5 GHz, 5G signal. The aim is to ensure consistency and compliance of the bioelectromagnetic experiments with good laboratory practices.

## 2. Equipments and Materials

- Radiofrequency (RF) generator (Rohde and Schwarz, SMW200A) with SMW-K144 and SMW-K148 options enabled to generate 5G compliant signal
- One wideband power sensor (Rohde and Schwarz, NRP-Z85)
- One directional coupler (Marki Microwave, CA40)
- Two customized stirred reverberation chambers (RCs) characterized by the electromagnetic perspective by University of Cassino (UCAS)
- Two customized stirrer controllers driving 2 stirrers for each RC, positioned on the right (M-Destro) and the bottom (M-Sotto) walls
- Two customized polystyrene stands for cell culture dishes
- Two WR-28 open-ended waveguides

- Two coaxial cables (PL 380P-292M292M-1M)
- PC for remote control through the Power Viewer program (Rohde and Schwarz)
- Two cell culture incubators (Thermo Scientific Forma, Model 311)
- 35 mm cell culture dish (Corning, cod. 430165)

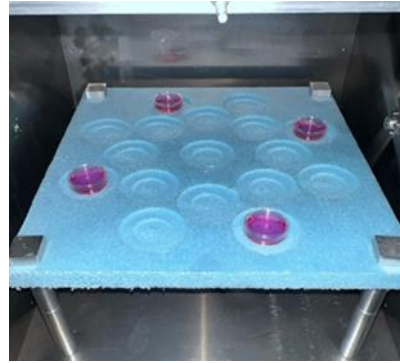


**Figure 1. Exposure system setup.** The signal is sent to the RC placed inside a cell culture incubator while a second RC, placed inside another cell culture incubator, is used for sham exposure. Each chamber is equipped with two stirrers at independently controlled speed and hosts a sample holder to allow reproducible positioning of the cell culture dishes.

### 3. Procedure

#### 3.1. Preparation of cell samples

- Prepare the following samples from the same batch of cells: incubator control, sham control, RF 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
- Locate the dishes on the stands (figure 2) placed inside the RCs for exposure and sham exposure and close each RC with the metallic slabs. Perform this procedure three hours before starting exposure to allow the sample to acclimate in the RCs



**Figure 2. Sample positioning in the RC.** The stand is opportunely engraved to allow a reproducible positioning of dishes.

### 3.2. RF exposure

- Turn on the generator at least 15 minutes before the start of exposure to allow warm-up
- Activate the 5G modulation (Baseband command enabled)
- Set the desired power level in dBm (set 2.66 dBm or 6.64 dBm to expose the samples at SAR 0.4 W/kg or SAR 1 W/kg, respectively)
- Turn on the stirrers in both RCs and set 240 for “M-Destro” and 233.3 for “M-Sotto”
- Turn on the PC and start the Power Viewer
- Connect the power sensor to the USB port of the PC
- Select the "Multi Channel" window. Channel 1 is turned off (Ch1: OFF). Move to channel 2 associated to the power sensor 2 (NRP-Z85, 102535) and set up the exposure conditions in the GUI (figure 3):
  - Frequency: 26.5 GHz
  - Averaging: manual
  - Count: 1048576
- Click on "ZERO" (with RF off) to start zeroing of the power sensor
- Click on the start button (on the top left of the toolbar) to start the visualization of the acquired data
- Select the "Data Log" window and check the following parameters:
  - Source: Multi
  - Ch1: OFF
  - Ch2: Ch 2 [W]
- Tick "Convert to Log Power," define the duration of the acquisition, tick "Log to File," set 500 ms in the "Interval" field and select the PC folder to save the data. Check that the value in the "Signal Frequency" field matches the value set on the generator (26.5 GHz)
- Click on the start button (in data log) to start data logging two minutes before the start of the exposure
- Start the RF manually from the generator
- At the end of the exposure, stop the RF manually from the generator

- Open the RCs
- Carefully remove the culture dishes from the stands
- Close the “experiment” program, switch off the PC and all the instruments
- Proceed with the harvest and the procedure for the biological assay by following the related SOP

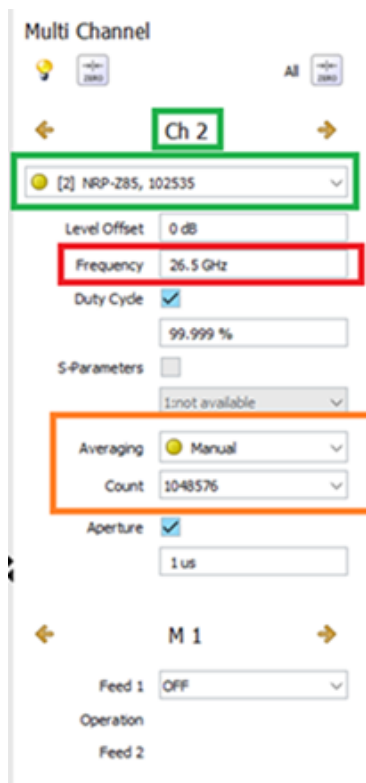


Figure 3. GUI of the Power Viewer program for the control of the exposure setup.