

Standard Operating Procedure @ CNR- IREA

PROTOCOL	Cell cultures simultaneous exposure to 4G LTE signal at 1.95 GHz and WiFi signal at 2.45 GHz
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1. Purpose

This procedure describes the equipment and the protocol used for cell cultures simultaneous exposure to electromagnetic field (EMF) at 1950 MHz (4G LTE signal) and at 2450 MHz (WiFi signal). The aim is to ensure consistency and compliance of the bioelectromagnetic experiments with good laboratory practices.

2. Equipment and Materials

- One RF generator (Agilent, E4432B ESG-D series)
- One RF generator (Rohde and Schwarz, SMM100A) with IEEE 802.11 option enabled to generate WiFi signal
- One power combiner (PD2020 InNSTOCK wireless components, NJ, USA)
- One microwave amplifier (MALTD, AM38A-0925-40-43)
- One power sensor (Rohde & Schwarz, NRTZ43)
- Two rectangular, short-circuited waveguides (WR430, 350mm long, SAIREM)
- Two coaxial-to-waveguide adapters (Maury Microwave R213A2; VSWR: 1.05)

- Four coaxial cables (SUHNER, SUCOFLEX SN233634 /4)
- PC for remote control of the power sensor through the R&S virtual NRT program (Rohde & Schwarz)
- One cell culture incubator (Thermo Scientific Forma, Model 311)
- 30 mm customized Pyrex cell culture dish
- Two four-layer customized plexiglass stands
- Two metallic slabs
- One plastic spacer

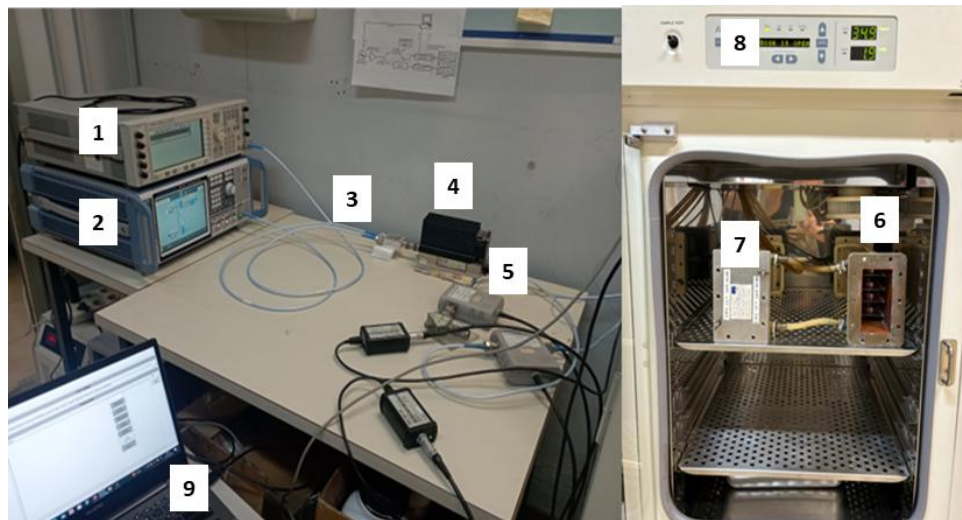


Figure 1. Exposure system setup. (1) Agilent RF generator, (2) Rohde and Schwarz RF generator, (3) power combiner, (4) microwave amplifier, (5) bi-directional power sensor, (6) waveguide for RF-exposure, (7) metallic slab for short-circuit, (8) cell culture incubator, (9) PC for remote control.

3. Procedure

3.1. Preparation of cell samples

- Establish the following samples from the same batch of cells: incubator control, sham control, RF exposed at two SAR levels (0.3 and 1.25 W/kg), 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 Plexiglas stands (figure 2), insert the stands in each waveguide at the required distance from the short-circuit by using the spacer and close each waveguide with the metallic slab. Perform this procedure two hours before starting exposure to allow the samples to acclimate in the waveguides.

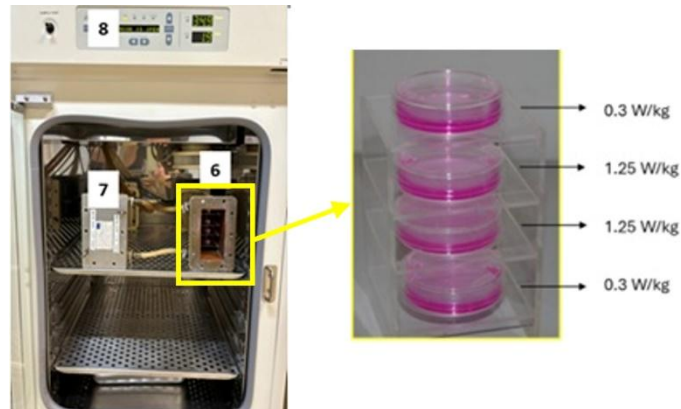


Figure 2. Plexiglas stand hosting cell culture dishes. A higher SAR value is obtained in the central samples with respect to the distal ones (4:1 SAR ratio).

3.2. RF exposure

- Switch on both RF generators and the PC
- (Optional) Open the NI-MAX program to verify that the generators and the power meter are detected
- Connect to the Wi-Fi network
- Use the Matlab code to generate the LTE signal: launch Matlab 2019 on the PC and open the LTE LOADER program by following the path "Desktop→ Esposizione 4G→ LTE LOADER_ versione_1.0"
- Enter "LTE_LOADER_v1p0" and follow the instructions to build the signal
- Disconnect from the Wi-Fi network
- Set the frequency (1.95 GHz) and the amplitude (−32 dBm) on the generator for 4G signal and verify that modulation is on (Mod ON)
- Set the frequency (2.45 GHz) and the amplitude (−32.6 dBm) on the generator for WiFi signal and verify that modulation is on (Mod ON)
- Start the R&S virtual NRT program.
 - Click on the "sensor" field to verify that the right power meter is detected, then click on "ZERO" to start zeroing of the power meter
 - Click on the "option" field, then on "record function" to set the separator (COMMA) and the Index (1)
 - Define the file name and browse the PC folder in which to save it
 - Click on "Auto Trigger" and set the START TIME (5 minutes before starting the RF), the STOP TIME (5 minutes after stopping the RF) and the interval (30 sec). Click on "enter" to verify the set parameters, then select "close"
- Start the RF manually from both the generators
- At the end of the exposure, stop the RF manually from both the generators
- Carefully remove the stands with the culture dishes from the waveguides
- Switch off the PC and all the instruments
- Proceed with the harvest and the procedure for the biological assay by following the related SOP