
	Protocol for Cell Exposure to a 3.5 GHz Electromagnetic Field	
		Version 01 du 29/04/2025 Auteur : E.Pinot
		Page 1 sur 3

1. Objective

This protocol aims to evaluate the biological effects of exposure to a **3.5 GHz electromagnetic field (RF)** on two different cell types:

- **Primary rat astrocytes** (Gibco),
- **Human SH-SY5Y neuroblastoma cells**.

The experimental design includes:

- **Two exposure durations:** 1 hour (short) and 24 hours (prolonged),
- **Three SAR levels:** SHAM (0 W/kg), 0.08 W/kg (low), 4 W/kg (high),
- **Two post-exposure analysis times:** **immediately** after exposure or **24 hours later (D+1)**.

2. Cell Material and Culture Conditions

Cell types:

- **Primary rat astrocytes** – Gibco (Thermo Fisher Scientific),
- **SH-SY5Y human neuroblastoma cells** – ATCC.

Culture support:

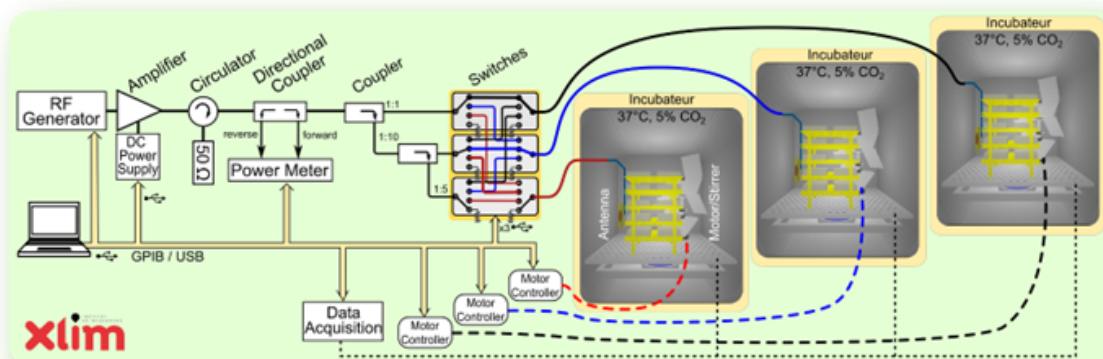
- **6-well plates**, compatible with the reverberation chambers,
- Each well contains **2 mL of culture medium**.

Seeding and incubation:

- Cells are **seeded 48 hours before exposure** to reach **70–80% confluency** at the time of the experiment.
- Cultures are maintained at **37°C**, under **5% CO₂** in a humidified atmosphere.

3. Exposure System and Conditions

- **Frequency:** 3.5 MHz, 5G modulation.
 - **Exposure system:**
 - **Three independent reverberation chambers** are used simultaneously, one chamber per SAR level.
-



Schematic of the exposure set up at 3.5 GHz

- **Specific Absorption Rate (SAR) levels:**
 - **SHAM:** 0 W/kg (no RF emission),
 - **Low SAR:** 0.08 W/kg,
 - **High SAR:** 4 W/kg.
- **Exposure durations:**
 - **1 hour** or **24 hours**, depending on the experimental condition.

4. Experimental Design



Cell Type	SAR (W/kg)	Exposure Duration	Analysis Time	Replicates
Astrocytes (Gibco)	0 / 0.08 / 4	1 h / 24 h	Immediate / D+1	n = 6
SH-SY5Y (human)	0 / 0.08 / 4	1 h / 24 h	Immediate / D+1	n = 6

5. Post-Exposure Biological Analyses

Cells are analyzed by **flow cytometry**, either **immediately** after RF exposure or **24 hours later** (D+1), depending on the condition.

Assays performed:

- **Mitochondrial oxidative stress:**
 - Detection using **MitoSOX Red** (mitochondrial ROS marker).
- **Apoptosis:**
 - Staining with **Annexin V** (early/late apoptosis and necrosis).
- **Cell viability:**

	Protocol for Cell Exposure to a 3.5 GHz Electromagnetic Field	
		Version 01 du 29/04/2025 Auteur : E.Pinot
		Page 3 sur 3

- Assessed by **Sytox blue**.
- **Cell proliferation:**
 - Tracked using **CellTrace™ Violet**

Each sample is analyzed for a minimum of **10,000 events**, with **6 biological replicates per condition** to ensure statistical robustness.