Jun 03, 2025

Low-frequency Mechanical Vibration Inducing Apoptosis In Vitro Cancer Cells

DOI
https://dx.doi.org/10.17504/protocols.io.81wgbk3d1gpk/v1

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Protocol Citation: Paola Di Maio 2025. Low-frequency Mechanical Vibration Inducing Apoptosis In Vitro Cancer Cells. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgbk3d1gpk/v1

Protocol status: Working
We use this protocol and it's working.

Created: June 03, 2025

Last Modified: June 03, 2025

Protocol Integer ID: 219457

Keywords: mechanical vibration, frequency mechanical vibration, vibration, cancer cell apoptosi, vitro cancer cells protocol, vitro cancer cells protocol for replication study, apoptosi, inducing apoptosi, mechanomedicine, cancer, damaging healthy tissue, cell

Disclaimer

Abstract

Protocol for replication study researching mechanisms to
induce cancer cell apoptosis without damaging healthy tissue.
The protocl describes how mechanical vibration, (mechanomedicine)
applied for one hour at brief low-frequency (20 Hz) mechanical vibration on
glucose consumption and survival (apoptosis, necrosis, HMGB1 release) results in
significantly increased at 48 h after mechanical vibration compared with cells maintained in static culture.

Materials

Main Protocol Materials

- Cell Line:
  - Human epidermoid carcinoma cell line

- Cell Culture Medium:
  - MEM/DMEM with:
    - 3.7 g/L sodium bicarbonate
    - 4.5 g/L glucose
    - 4 mM glutamine
    - 10% fetal bovine serum (FBS) for initial culture
  - Serum-free DMEM for 48 h prior to experiments.

- Mechanical Vibration System:
  - Vibration transducer or other equipment capable of delivering sinusoidal wave with or without contact
  - Controller software: Pd-extended on PC
  - Vibration frequency: 20 Hz sinusoidal waves

- Assay Kits:
  - Apoptosis/Necrosis Detection Kit
  - Glucose Assay Kit
  - HMGB1 ELISA Kit

- Microscopy and Imaging:
  - Eclipse Ti-S inverted microscope
  - NIS-Elements D software for image capture
  - ImageJ software for quantification.

- Incubation Conditions:
  - 37°C, 5% CO2 for culture
  - Vibration performed at 34°C inside humidified CO2 incubator, medium temperature reaches 37°C during vibration.

Protocol Steps

1 Cell Preparation:

1.1 Seed cells at 1.5 × 10^5 cells per 250 µL well in 12 central wells of a 96-well plate.

1.2 Culture for 48 h in serum-free medium at 37°C, 5% CO2.

2 Mechanical Vibration Application:

2.1 Place the 96-well plate on the vibration transducer secured with double-sided tape.

2.2 Apply mechanical vibration at 20 Hz sinusoidal frequency for 1 hour inside a humidified CO2 incubator at 34°C (medium reaches 37°C during vibration).

3 Post-Vibration Incubation:

3.1 Remove plates from vibration system.

3.2 Incubate cells without vibration for 0 (immediate), 24, or 48 hours at 37°C, 5% CO2.

4 Apoptosis and Necrosis Assay:

4.1 At 0, 24, and 48 hours post-vibration, analyze cell death using Apoptosis/Necrosis Detection Kit.

4.2 Visualize cells by inverted microscopy and quantify apoptotic and necrotic areas using ImageJ.

5 Glucose Consumption Assay:

5.1 Collect culture supernatants at indicated times.

5.2 Measure glucose concentration using Glucose Assay Kit following manufacturer's protocol.

6 HMGB1 Release Measurement:

6.1 Collect and pool supernatants at indicated time points.

6.2 Perform HMGB1 ELISA according to manufacturer's instructions, including coating, washing, antibody incubations, and colorimetric detection at OD450.

7 Data Analysis:

7.1 Perform experiments in biological triplicates.

7.2 Present data as mean ± SD.

7.3 Use Student's t-test for group comparisons; significance at $p < 0.05$.

Protocol references

Anggayasti WL, Imashiro C, Kuribara T, Totani K, Takemura K. Low-frequency mechanical vibration induces apoptosis of A431 epidermoid carcinoma cells. *Eng Life Sci* 2020; 20: 232–238. https://doi.org/10.1002/elsc.201900154