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Senescence induction by ionizing radiation in human PCLS

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We use this protocol and it's working

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Abstract

Precision-cut lung slices (PCLS) are standardized human lung tissue sections that serve as an advanced ex vivo platform for studying disease mechanisms, screening therapeutic compounds, and supporting preclinical research, while preserving native cellular diversity and structural organization of the lung.

This protocol outlines an method for inducing cellular senescence in PCLS using ionizing radiation, along with procedures for harvesting tissue samples for downstream analyses and quantitative assessments.

Materials

- Cultivation medium (DMEM/F12, 11330057)
-FBS Good (PAN Biotech P40-37500)
-Pen-Strep (Life technologies 15140122)
-Amphotericin B (Sigma A2942-100ML)
-DPBS 1X (Gibco, 14190-094)
-Paraformaldehyde solution 4% in PBS (Santa Cruz sc-281692)
-Machine: RS225 X-ray cabinet (Xstrahl, Camberley, UK)

Before start

To start this procedure, you must have PCLS (Precision-Cut Lung Slices) ready.

Day -1: PCLS acclimation to growth conditions

1 Add 100 ul of cultivation media per well in 96-well plates in cultivation medium (DMEM-F12 supplemented with 0.1% FBS, 1% Pen-Strep and 1% amphotericin B)

2 Place one PCLS (4mm / 500 µm thick) per well.

3 Incubate o/n (overnight) at 37ºC, 5% CO2.

Day 0: Medium change

4 Warm up cultivation medium to 37ºC.

5 Inside a cell culture hood, remove medium from all wells.

6 Add fresh cultivation medium (100ul/well)

Day 0: Ionizing radiation

29m 24s

7 Transfer 96-wells to the room for radiation. Use a transport box.

8 Switch the machine on and log in.

9 Clean the machine with EtOH.

10 Press "X-Ray"

11 Close the appliance door manually.

12 Press “warm-up” → “Short” → Confirm” 17m

13 Turn the key from "Stand-by" to "X-Ray.”

14 The start button now lights up green à Device ready à Press the button à Warm-up begins.

15 The Warm-up is done after 17 minutes.

16 Turn the key from "X-Ray" to "Stand-by" à only now does the appliance door open again.

17 Load the device with samples (inner circle)

18 Close the door manually.

18.1 Set up exposure times accordingly with these parameters: 12m 24s
195 KV / 15 mA. Calculate desired dose: 2.42 Gy /minute à 1 Gy = 24.8 seconds
30 Gy: 12 min 24 sec

19 Press "confirm"

20 Turn the key from "Stand by" to "X-Ray".

21 The start button now lights up green à Device ready à Press the button à X-Ray begins.

22 Turn the key from "X-Ray" to "Stand-by" à only now does the appliance door open again.

23 Transfer 96-well plates back to cell culture incubator.

Day 0- Day 7: Culture and samples collection

1h 30m

24 PCLS can be kept in culture for up to 7 days. Change medium every 2-3 days.

25 Collect PCLS on day 1, 3, 7 (sterile conditions):

Supernatant Collection

1h 30m

25.1 Transfer supernatants to autoclaved 2ml Eppendorf tubes

25.2 Spin down at 1000 rpm at 4ºC.

25.3 Transfer supernatant to new tubes and freeze down at -80ºC.

Tissue Frezing for RNA isolation

1h 30m

25.4 Wash punches once with 1X DPBS.

25.5 Transfer 6 punches per condition into RNAse-free 2ml Eppendorf tubes.

25.6 Drop eppis tube in liquid N2.

25.7 Transfer to -80ºC.

Tissue fixation

1h 30m

25.8 Wash punches once with 1X DPBS.

25.9 Fix hPCLS with 4%PFA at 37ºC for 1 hour or at 4ºC overnight.

25.10 Wash 3X with 1X PBS and keep in 1X PBS at 4ºC until use.