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Senescence induction by DNA-damage in PCLS

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Cellular Senescence Net...



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We use this protocol and it's working

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Abstract

Precision-Cut Lung Slices (PCLS) are uniform, in size and thickness, tissue slices generated from human lungs that can be used for disease modeling, drug discovery and preclinical validation, while retaining the lung's cellular complexity and tissue architecture.

This protocol describes two different methods for the induction of cellular senescence by DNA damage in PCLS, and collection of samples for different analysis and measurements.

Image Attribution

CLAR (Center for Lung Aging and Regeneration) Logo designed by Nayra Cardenes, PhD.

Guidelines

All steps involving human tissue must be conducted under BSL2 safety guidelines.



Protocol materials

- ✕ 16% Formaldehyde (w/v), Methanol free **Life Technologies Catalog #28908**
- ✕ WST-8 Assay Kit (Cell Proliferation) **Abcam Catalog #ab65475**
- ✕ Senescence β -Galactosidase Staining Kit **Cell Signaling Technology Catalog #9860**
- ✕ Bleomycin **Fresenius Kabi Catalog #10361**
- ✕ Dimethyl sulfoxide (DMSO) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D2438-50ML**
- ✕ Doxorubicin hydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D1515-10MG**
- ✕ DMEM/F-12, powder, HEPES **Thermo Fisher Scientific Catalog #12400024**
- ✕ Penicillin-Streptomycin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P00781**
- ✕ Amphotericin B solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A2942**

Safety warnings

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Before start

To start this procedure, you must have PCLS (Precision-Cut Lung Slices) ready (check protocol DOI dx.doi.org/10.17504/protocols.io.36wgq3xr5lk5/v1).

Day -1: PCLS acclimation to growth conditions

- 1 Add 1 mL of media per well in 24-well plates DMEM F-12 media ( DMEM/F-12, powder, HEPES **Thermo Fisher Scientific Catalog #12400024**) + 1% FBS + 1% Penicillin-Streptomycin  Penicillin-Streptomycin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P00781** + 0.3 µg/mL Amphotericin B  Amphotericin B solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A2942** .
- 2 Place one PCLS (1 cm diameter / 300 µm thick) per well with a brush.
- 3 Incubate o/n (overnight) at 37 °C, 5% CO₂.

Treatment at Days 0, 2 and 4

- 4 Treatment: change media with 1ml/PCLS, diluted in DMEM media + 0.1% FBS + 1% Pen/Strep + 1% Amphotericin B
- 4.1 Prior to media exchange, collect supernatant (s/n) from each condition in 1.5 mL tubes and, store at -80°C for future analysis.
- 4.2 Change media for each condition:

Control - Change media (above).
Bleomycin - 15 µg/ml Bleomycin in media above.  Bleomycin **Fresenius Kabi Catalog #10361**

DMSO - 1:100,000 DMSO in media above.  Dimethyl sulfoxide (DMSO) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D2438-50ML**

Doxorubicin - 0.1 µM Doxorubicin in media above.  Doxorubicin hydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D1515-10MG**

PCLS Harvesting for measurements



- 5 Collect PCLS at Day 0, 2, 4 and Day 6 for different baseline and final timepoint measurements.
- 5.1 Days 0, 2, 4 6:
Fix PCLS in 4% formaldehyde/PBS for 30 minutes (2x PCLS per condition).
 16% Formaldehyde (w/v), Methanol free **Life Technologies Catalog #28908**
Then wash twice with PBS and store at 4°C in PBS.
Use 1-2 slices for paraffin embedding, and posterior histological analysis.
- 5.2 Days 0 and 6:
Cell proliferation measurement: Use 2-3x PCLS to make 4×4 mm punches to perform WST-8 cell proliferation assay in a 96-well plate, following manufacturer's instructions.
 WST-8 Assay Kit (Cell Proliferation) **Abcam Catalog #ab65475**
- 5.3 Days 0 and 6:
Fix 2x PCLS with 1x fixative solution from β-galactosidase kit and stain for β-galactosidase as per manufacturer instructions.
 Senescence β-Galactosidase Staining Kit **Cell Signaling Technology Catalog #9860**
- 5.4 Day 0 and 6:
Snap freeze in liquid nitrogen: 4x PCLS per cryotube.
Store at -80°C for future analysis.