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Senescence induction by DNA-damage in PCLS

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Protocol status: Working
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

- Penicillin-Streptomycin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P00781 Step 1
- Amphotericin B solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A2942 Step 1
- WST-8 Assay Kit (Cell Proliferation) **Abcam Catalog #**ab65475 Step 5.2
- Bleomycin **Fresenius Kabi Catalog #**10361 Step 4.2
- Dimethyl sulfoxide (DMSO) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D2438-50ML Step 4.2
- Doxorubicin hydrochloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D1515-10MG Step 4.2
- DMEM/F-12, powder, HEPES **Thermo Fisher Scientific Catalog #**12400024 Step 1
- 16% Formaldehyde (w/v), Methanol free **Life Technologies Catalog #**28908 Step 5.1
- Senescence β-Galactosidase Staining Kit **Cell Signaling Technology Catalog #**9860 Step 5.3

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 ^{o}C, 5% CO_2.

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^{o}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^{o}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 4x4 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^{o}C for future analysis.