CAMPISI LAB PROTOCOL FOR DETECTING SENESCENCE-ASSOCIATED B-GALACTOSIDASE (SA-Bgal) ACTIVITY
OR . . . HOW TO TURN YOUR OLD CELLS BLUE

REFERENCE: Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I, Pereira-Smith O, Peacocke M, Campisi J. (1995) A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc. Natl. Acad. Sci. USA 92: 9363-9367.

For Cells on Tissue Culture Dishes or Slides:

- Wash cells 2X with PBS
- Fix 3-5 min, room temperature, with 2% formaldehyde + 0.2% glutaraldehyde in PBS.

Note: 3% formaldehyde also works, but the above preserves morphology somewhat better.
DO NOT OVERFIX – 5 MIN MAXIMUM; REDUCE TIME IS STAINING IS WEAK

- Wash cells 2-3X with PBS.
- Add Staining Solution (1-2 ml per 35 mm dish, or immerse slide).
- Incubate at 37° C - ***NOT in a CO_2 incubator***.

Blue color is detectable in some cells within 2 h, but staining is usually maximal in 12-16 h.

Staining Solution:

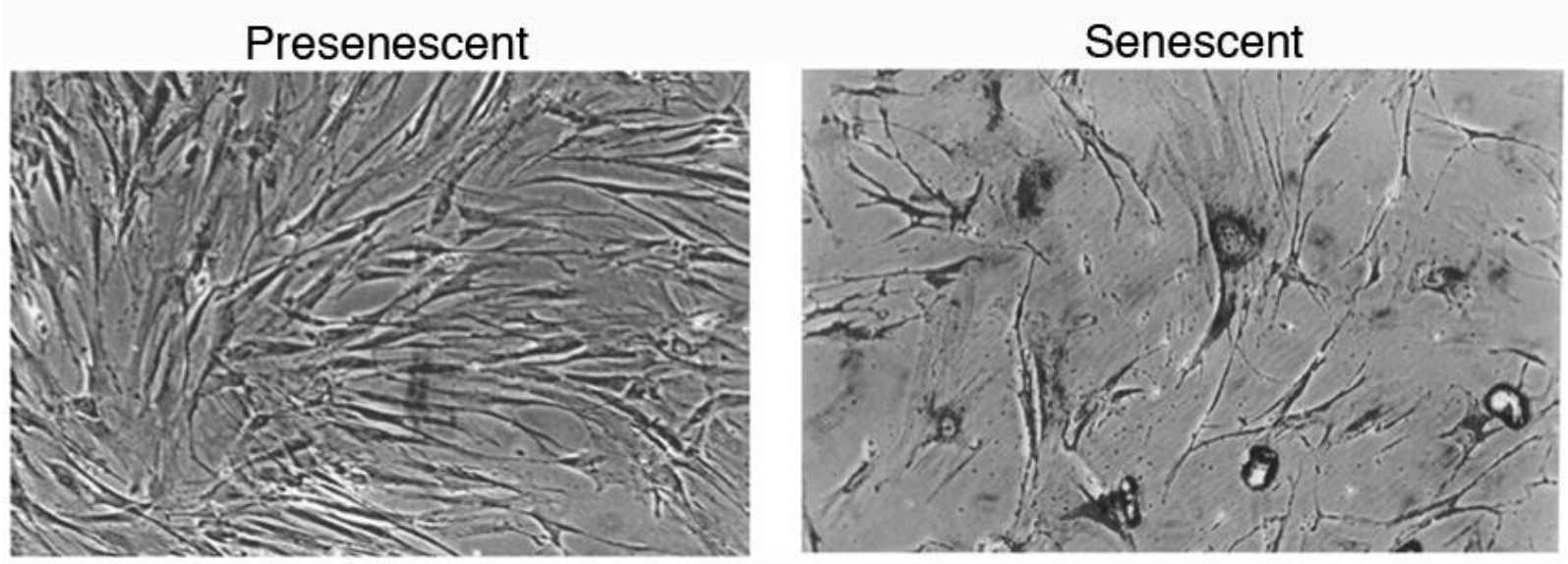
Component	Volume	Final Concentration
20 mg/ml X-gal in dimethylformamide	1 ml (*See Note*)	1 mg/ml
0.2M citric acid/Na phosphate buffer, pH = 6.0 (***pH is important***!)	4 ml	40 mM
100 mM potassium ferrocyanide	1 ml	5 mM
100 mM potassium ferricyanide	1 ml	5 mM
5 M sodium chloride	0.6 ml	150 mM
1 M magnesium chloride	0.04 ml	2 mM
Water	12.4 ml; Total volume = 20 ml	

Citric acid/Na phosphate buffer, 0.2M (100 ml):
36.85 ml 0.1 M citric acid solution
63.15 ml 0.2 M sodium phosphate (dibasic) solution
Verify that pH is 6.0.

Citric acid solution (0.1 M) = 2.1 g citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$)/100 ml water
Sodium phosphate solution (0.2M) = 2.84 g sodium dibasic phosphate (Na_2HPO_4) or 3.56g sodium dibasic phosphate dihydrate ($Na_2HPO_4 \cdot H_2O$) (be sure to use dibasic)/100 ml water

Note: Store X-gal solution at -20° C. Buffer and salts may be prepared several days or weeks ahead of time. However, X-gal is not stable in aqueous solution, so add it the day of the assay, no earlier.

How Should It Look?



Combined ^{3}H-Thymidine Autoradiography and SA-Bgal Staining:

Label cells with ^{3}H-thymidine. We use 10 µCi/ml (50-80 Ci/mmol) for 48-72 hrs.
Wash cells 2X with PBS, fix and stain for SA-Bgal activity, as described above.
After the color develops, wash cells 2X with PBS, 2X with methanol and air dry.
Coat the cells with Kodak NTB2 emulsion, store in the dark 12-48 h, develop (we use D19 or Microdol X), fix (we use Kodak Rapid-Fix), and rinse well with water.

FOR TISSUE SECTIONS

Our collaborators tell us the above protocol can be used for fresh frozen tissue sections, but it is CRUCIAL that the tissues be flash frozen in liquid nitrogen, immediately imbedded in OCT, sectioned (4 micron sections recommended), mounted onto slides, then fixed and stained. **Overnight storage of tissue samples at -80º C can destroy the enzyme activity in some cases**. Fixation and staining conditions may vary, depending on the tissue. For human skin, best fixation condition was 1% formaldehyde in PBS for 1 min, wash with PBS, followed by overnight incubation with staining solution.