Protocol for SA-β-Galactosidase assays on frozen tissues slides

Solutions:

- **Fixation solution:** 0.2% Glutaraldehyde in PBS 1X
- **Permeabilisation solution:** 0.02% NP-40 (*aka* Igepal), 0.01% Sodium Deoxycholate, and 2mM $MgCl_2$ in 0.1M phosphate buffer pH 6.0 *(See recipe below)*
- **X-Gal staining solution:** 0.02% NP-40 (*aka* Igepal), 0.01% Sodium Deoxycholate, KC Solution 1X *(See recipe below)*, 2mM $MgCl_2$, and 1mg/mL X-Gal* (diluted in N,N-dimethylformamide) in 0.1M phosphate buffer pH 6.0 → Incubate the solution at 37°C for 10 mins and then filter with a 0.2µm, before using it.
  *From stock at 40X (40mg/mL)
- 4% **Paraformaldehyde** (PFA)
- **Haematoxylin** diluted 1/10 in ddH_2O
- **Mounting media:** CytoSeal
- **OCT** Compound (Sakura)

Recipes:

- **Phosphate Buffer:**

Preparation of 0.1M Sodium Phosphate Buffer at 25°C		
pH	**Volume of 1M Na_2HP0_4 (mL)**	**Volume of 1M NaH_2P0_4 (mL)**
5,8	7,9	92,1
6,0	12,0	88,0
6,2	17,8	82,2
6,4	25,5	74,5
6,6	35,2	64,8
6,8	46,3	53,7
7,0	57,7	42,3
7,2	68,4	31,6
7,4	77,4	22,6
7,6	84,5	15,5
7,8	89,6	10,4
8,0	93,2	6,8
Then, DILUTE the combine 1M stock solutions to 1L with ddH_2O		

- **KC Solution 20X:**

KC (20X stock)							
Potassium Ferricyanide:	**$K_3Fe(CN)_6$ (g)**	0,82	1,64	3,28	4,92	6,56	**(g)**
Potassium Ferrocyanide:	**$K_4Fe(CN)_6$ x 3H2O (g)**	1,05	2,1	4,2	6,3	8,4	**(g)**
	In PBS, final volume (mL)	25	50	100	150	200	**(mL)**

N.B. : At 1X, Potassium Ferricyanide is at 6.5mM and Potassium Ferrocyanide at 5mM

Procedures:

N.B. Do not use *PapPen* for this protocol as it reacts with KC solution, forming ferrous precipitates on samples!

Day 1:

1. Perform perfusion on mice with 10mL sterile PBS 1X and harvest tissues of interest.
2. Freeze tissues individually in OCT on dry ice/isopentane bath.
3. Cut 10µm slides from frozen blocks with microtome, and store cut slides at -80°C until staining.
4. When ready for assay, remove slides from -80°C and fix them in **Fixation solution** for 10 mins at 4°C.
5. Rinse once with PBS 1X, then wash in PBS 1X for 10 min.
6. Wash in **Permeabilisation solution** for 10 min at room temperature.
7. Incubate with **X-gal staining solution** overnight at 37°C in the dark.

Day 2:

8. Fix slides in **4% PFA** for 10 mins at room temperature.
9. Rinse once with PBS 1X, then wash in PBS 1X for 10 mins.
10. Wash 2 x 5 mins in ddH_2O, and counter-stain with **Haematoxylin** diluted 1/10 for 40 secs.
11. Rinse once with ddH_2O, then wash in ddH_2O for 2 mins.
12. Go to microscope to see if enough stained. If so, go to step 13. If not, stain again with Haematoxylin, then stop staining by washing 2 mins with ddH_2O.
13. **Dehydrate** following these steps (~250mL solution per bath):

Steps	Solutions	Time
1	EtOH 40%	1 x 1 min
2	EtOH 75%	1 x 1 min
3	EtOH 95%	1 x 1 min
4	EtOH 100%	1 x 1 min
5	Xylene	2 x 5 mins

14. Remove from last Xylene bath and let it dry under the chemical hood for ~45 mins.
15. Mount slides with ***CytoSeal*** and add coverslip.
16. Let it dry overnight at room temperature and image slides.