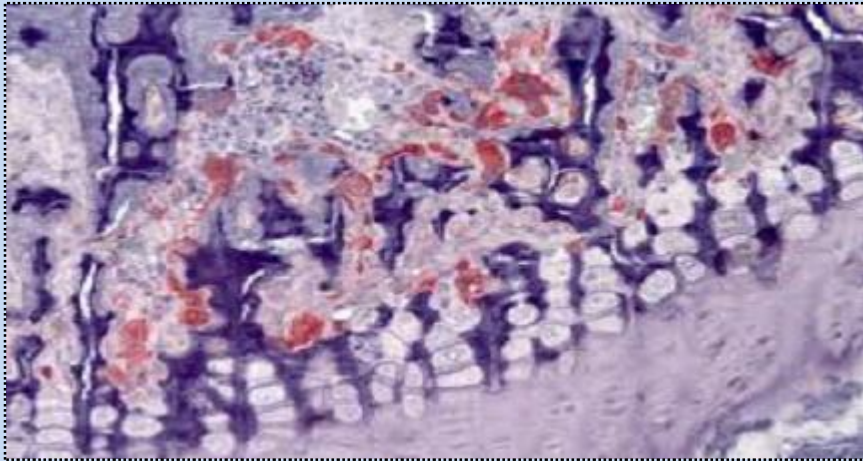


Protocol for TRAP staining



1. Preheat to 37°C 2 Coplin jars with 50ml of Buffer I Solution each.
2. Take one Coplin jar and add 0.5 ml of Buffer II Solution, add slides and incubate at 37°C for 45 minus.
3. A few min before the time is up, mix 1 ml of Buffer III Solution and 1 ml of Buffer IV solution for 30 sec and let it sit for 2 min.
4. Add this mixed solution to the other Coplin jar of 50 ml Buffer I Solution, mix and add the slides without rinsing.
5. Incubate at room temp ~5 min.
6. Rinse, counterstain with hematoxylin for 40 sec and put slides in 0.05% Ammonia water.
7. Dehydrate through alcohol, clear in xylene and mount.