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### **Procedure for Alkaline and Fpg-modified Alkaline Comet Assay**

1. Thaw the frozen blood in a 37 °C water bath and put on ice.
2. Mix blood suspension with LMA at a ratio of 1: 20 (i.e., 5 µl blood suspension per 100 µl LMA).
3. Add 70µl of cell-agarose solution to center of each slide well and spread around using pipette tip.
4. Store slides prepared during the above steps @ 4°C for 40 minutes to allow agarose to adhere to slides
5. Carefully immerse slides in Lysis Buffer in the dark @ 4°C for one hour to lyse cells
6. Wipe/tape off excess Lysis Buffer from slides.
7. For Alkaline comet assay, continue from “step 12”.
8. Immerse slides Fpg buffer in the dark @ 4°C for 5 min.
9. Repeat 4) for 2 times.
10. Dilute Fpg stock using Fpg buffer (Dilution factor will depend on the activity of the enzyme and the type of cells).
11. Add 100 µl Fpg or buffer to each well, incubate the slides at 37 °C for 20-60 min.
12. Immerse slides in alkaline buffer in the dark at 4°C for 40 min.
13. Electrophoresis in the dark @ 4°C for 30 minutes @ 25V, 300mA in Running Buffer.  
(Use the amounts of buffer to adjust the current)
14. Place slides in a **glass holder** and gently immerse with the Tris Buffer in the dark @ 4°C for five minutes
15. Immerse slides in DI H<sub>2</sub>O in the dark @ 4°C for 5 minutes
16. Repeat Step 11 once.
17. Air dry slides in the dark @ room temperature overnight
18. Store slides @ 4°C until analysis
19. Stain slides by placing 25µl of 20µg/ml ethidium bromide, then cover with cover glass.
20. Score 50-100 cells under a fluorescent microscope using Komet 5.5 software. Use Olive Tail Moment to quantitate DNA damage. It incorporates a measure of both the smallest detectable size of migrating DNA [reflected in the comet tail length] and the number of relaxed/broken pieces [represented by the intensity of the DNA in the tail]; DNA damage = tail length X DNA intensity in tail.

21. To obtain oxidative DNA damage, subtract the average value of Olive tail moment of Fpg treated cells from buffer control.