

FISH SLIDES

Fix: 3 parts Methanol/ 1 part Glacial Acetic Acid

NOTE: The first time fix is added to the culture make sure the MeOH is very fresh !!

1. Spin fixed cells 5 min. at 1000 rpm in Beckman GP centrifuge.
2. Decant supernatant with Pasteur pipet leaving about 750 μ l of supernatant.
3. Add 7mL of new fix and resuspend the pellet by flicking the tube.
4. Spin cells 5 min. at 1000 rpm in Beckman GP centrifuge.
5. Decant supernatant with Pasteur pipet. Size of the pellet will determine the volume of supernatant left to resuspend the pellet.
6. To resuspend the pellet, put the end of a 9" Pasteur pipet in supernatant over the pellet and then squeeze the pipet rubber bulb to blow bubbles over pellet.
7. Use Superfrost precleaned Premium Microscope Slides from Fisher.
8. To clean the slides, place them in a Copelin jar containing 100% EtOH for a few minutes.
9. Remove slide from EtOH and dry with Kimwipe making sure no lint is left behind.
10. Wet the top of the slide with distilled water and then tap on paper towel to remove excess water.
11. Using a 5" Pasteur pipet and rubber bulb, bring the fixed cell suspension just up to the wide bore opening of the pipet.
12. One to two drops of the suspension is dropped onto the slide angled at 45° at least 6" from the tip of the pipet.
13. Depending on weather conditions the slide may be placed over steam for 10sec. to spread chromosomes. Steam slides by placing over a boiling water bath.
14. Allow the slide to dry and view with the Phase Contrast microscope.