

## **VITALITY STAIN**

**TAKE A GLASS SLIDE & CLEAN IT BOTH THE SIDES**



**MAKE THE SEMEN SAMPLE READY FROM THE SAMPLE  
WARMER AT 37 DEGREE CELCIOUS**



**ADD SEMEN SAMPLE ABOUT 50 MICRO LITTER IN THE 1 ML  
TUBE & ADD 50 MICRO LITTER VITALITY STAIN**



**MIX WELL & INCUBATE IT FOR 2 MINITUES**



**TAKE 10 MICRO LITTER OF THIS MIXTURE**



**PLACE IT IN THE GLASS SLIDE ON THE RIGHT SIDE & MAKE A  
THICK SMEARE**



**DRY IT IN THE SLIDE WARMER**



**OBSERVE IT IN THE MICROSCOPE AT 40X OBECTIVE.**

## **MORPHOLOGY STAIN**

**CENTRIFUGE THE SEMEN SAMPLE FOR 3 MINUTES AT 1500 RPM  
& REMOVE THE SUPERNATANT**



**ADD WASH MEDIUM IN THE PELLET & CENTRIFUGE IT AGAIN IN  
THE SAME RPM( REPEAT THIS FOR 3 TIMES)**



**THEN REMOVE THE SUPERNATANT AGAIN & ADD MINIMUM  
AMOUNT OF WASH MEDIUM**



**THEN MIX WELL & TAKE 10MICRO LITER OF THIS SAMPLE**



**PLACE IT IN CLEAN GLASS SLIDE & MAKE A THICK SMEAR**



**DRY IT IN SLIDE WARMER & PLACE IT IN THE STAINING TRAY**



**ADD FIXATIVE-1 AND WAIT FOR 15 SECONDS & DRAIN IT**



**ADD STAIN-1 WAIT FOR 10 SECONDS & DRAIN IT**



**ADD STAIN-3 WAIT FOR 9 SECONDS & IMMEDIATELY WASH IT  
WITH DISTILL WATER**



**DRY IT WITH SLIDE WARMER UNTIL DRY**



**OBSERVE IT IN MICROSCOPE AT 100X OIL EMERSSION & COUNT  
100 SPERMS**

**DNA STAIN**

**PLACE THE GELL IN THE DNA WARMER AT 75° FOR 20 MINITUES**



**AFTER MELTING THE GELL PLACE IT AT 37°C SAMPLE WARMER FOR 5 MINITUES**



**ADD 60 µl OF WASHED SAMPLE IN THE GELL AND MIX WELL**



**WARM IT FOR 5 MINITUES IN 37°C**



**TAKE A DNA SLIDE & PLACE 15 µl OF THE GELL SAMPLE IN THE CENTER PART OF THE SLIDE & PLACE THE COVER SLIP WITH OUT BUBBLE**



**KEEP THE SLIDE AT 2° - 4° FOR 5 MINITUES. THEN REMOVE THE COVER SLIP GENTLY**



**PLACE THIS SLIDE IN THE IMMERSION TRAY & ADD 3 ML OF LYSE1 & KEEP IT FOR 7 MINITUES.**



**TAKE THE SLIDE AND PLACE IT IN THE SECOND POSSITION OF THE SAME TRAY & ADD 3 ML OF LYSE 2 . KEEP IT FOR 25 MINITUES.**



**TAKE & CLEAN THE BACK SIDE OF THE SLIDE PLACE IT IN THE 3<sup>rd</sup> POSSITION & ADD 5 ML DISTILLED WATTER , KEEP IT FOR 5 MINITUES**



**THEN PLACE THE SLIDE IN THE TRAY & COVER IT WITH  
FIXATIVE-1 FOR 2 MINITUES**



**DRAIN IT & ADD FIXATIVE-2 FOR 2 MINITUES FOLLOWED BY  
FIXATIVE-3 FOR 2 MINITUES & DRAIN IT .**



**ADD STAIN-1, 1 ML INCUBATE FOR 2 MINUTES. ADD BUFFER  
2ML WITH OUT DRAIN THE STAIN – 1 & KEEP IT FOR 15  
MINITUES**



**RINCE IT WITH DISTILLED WATER & DRY IT . OBSERVE IT IN  
40X OBJECTIVE**



**COUNT MINIMUM 100 - 300 SEMEN PER SLIDES.**

**FOR ANY CLARIFICATION PL CALL**

**BORUS  
TECHNICAL & MARKETING MANAGER  
9940046139**