Detection of Pan-MCM in Formalin-Fixed, Paraffin Embedded Rat Tissue Using the Ventana

Staining Procedure
-Positive Control Tissue: GI tract
-Stain Localization: Nuclear

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Cut 2 sequential 6 micron sections onto tissue adhesive slides (Daigger ? Vernon Hills, Illinois - Superfrost Plus Catalog #GX15978Z). Do not pre-heat treat the slides in histology. The slides should be allowed to air dry.

Deparaffinize and hydrate slides through the following solutions

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

2. Perform Heat Induced Epitope Retrieval using Microwave Oven.
   Place a full rack of slides in Tissue Tekô container containing 200 mls distilled water.

    MWO for 5 minutes at 50% power
    Cool for 1 minute (Add 50 mls distilled water to container)
    MWO for 5 minutes at 50% power
    Cool 20 minutes at room temperature
    Rinse in distilled water 3 X 2 minutes each

3. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

4. Place slides on Ventana

    Protocol # 76
     No Amplification
     A/B Block Master Lot #_____
     DAB Detection Kit Master Lot #_____
     Titration run ? 32 minute incubation

    Primary antibody : Polyclonal Rabbit anti-Human Pan-Minichromosome

Maintenance Protein (MCM)
BDPharmingen
San Deigo, CA 92121
www.bdbiosciences.com
1-877-232-8995
Catalog #559541
Lot #_____ Date received _____

1:300

Upon completion of run, remove slides from machine and rinse in warm detergent water to remove Ventana coverslip from slides.

5. Rinse in tap water 3 minutes.

6. Counterstain with Modified Harris Hematoxylin for 45 seconds.

7. Rinse in tap water until water is clear.

8. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.

9. Dehydrate through the following solutions.

1. 95% Ethanol	2. 1 change	3. 3 minutes
4. 100% Ethanol	5. 3 changes	6. 3 minutes
7. Xylene	8. 2 changes	9. 5 minutes

10. Coverslip.

last updated 4/15/2003