

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

For acetomeniphen samples

Reagents:

[1X Automation Buffer](#)

[3% Hydrogen Peroxide](#)

[Antibody Diluent](#)

[Citrate Buffer](#)

[DAB Chromagen](#)

Hematoxylin

Antibody Information:

Blocking Serum: Normal Goat Serum

Jackson Immunoresearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #005-000-001

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog #SP-2001

Primary antibody : Polyclonal Rabbit anti-Human Pan-Minichromosome Maintenance Protein (MCM)

BDPharminingen

San Deigo, CA 92121

www.bdbiosciences.com

1-877-232-8995

Catalog #559541

Negative control serum: Normal Rabbit Serum

Jackson Immunoresearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #011-000-001

Concentrator: 60 mg/ml

Secondary antibody: Biotinylated anti-rabbit IgG (made in goat)
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #BA-1000
Suggested dilution: 1:500

Label antibody: Vector EliteVectastain® ABC
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #PK-6100

Microwave Information
Panasonic High Power 1200W
Model number NN-S732WL
Conditions for retrieval dependent upon this microwave

Staining Procedure

- Positive Control Tissue: BRDU treated rat liver
- Stain Localization: Nuclear

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. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.
2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
3. Perform Heat Induced Epitope Retrieval using Microwave Oven.
Place a full rack of slides in Tissue Tek® container containing 200 mls distilled water.
MWO for 5 minutes at level 3
Cool for 1 minute (Add 50 mls distilled water to container)

MWO for 5 minutes at level 3Temp. _____
Cool 20 minutes at room temperature
Rinse in distilled water 3 X 2 minutes each
Place slides in buffer for 5 minutes

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

5. Block in 5% Normal Goat Serum for 20 minutes.

Lot# _____ Reconstituted Date _____

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

6. Apply Avidin/Biotin block

Lot# _____ Exp _____ New Kit yes / no

Apply avidin block - 15 min @ RT.

Quick rinse in 1X Automation Buffer. (Do not leave slides sitting in buffer)

Apply biotin block - 15 min @ RT.

No wash, wipe excess block and apply primary antibody

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Pan-MCM) at 1:250 dilution and incubate for one hour.

Lot# _____ Exp Date _____

1:250

For the negative control slides, match the protein concentration of the normal rabbit serum to the protein concentration of the primary antibody (Pan-MCM) and use this to make the 1:250 dilution. Apply to the slides and incubate for one hour.

Lot # _____ Reconstituted Date _____

1:250

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

9. Apply secondary antibody (Goat anti-rabbit) at 1:500 dilution and incubate for 30 minutes.

Lot # _____ Exp Date _____

1:500

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

11. Apply Label antibody and incubate for 30 minutes.

(Prepare at least 30 mins prior to use) 2 drops Reagent A + 5 ml diluent -> Mix and then add 2 drops Reagent B

Kit Lot# _____ Exp. Date _____ New kit yes / no

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

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.

(Add 1 drop of DAB per ml of substrate)

Lot# _____ Exp. Date _____ New kit yes / no

Rinse in tap water 3 minutes.

14. Counterstain with Modified Harris Hematoxylin for 30 seconds.

15. Rinse in tap water until water is clear.

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

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip

updated 4/9/2003
