

REF 005-11000-40

7 mL

CE

IVD



2°C 8°C

REAGENT DESCRIPTION

Clone MCM2 26H6.19, MCM2 27C5.6, TOP2A SWT3D1
Ig Class IgG₁
Immunogen Recombinant Human MCM2 and TOP2A

1. INTENDED USE

For In Vitro Diagnostic use.

For use with automated staining on the Ventana Benchmark[®] XT using the iView[™] detection chemistry.

The ProEx[™] C Immunohistochemical Test is intended for the qualitative evaluation of aberrant S-Phase induction in formalin-fixed paraffin-embedded tissue biopsies. Results interpretation must be made by a certified professional within the context of the patient's history and other diagnostic tests.

2. SUMMARY AND EXPLANATION

Minichromosome maintenance (MCM) and topoisomerase II alpha (TOP2A) proteins play an important regulatory role in eukaryotic DNA replication. For example, the HPV oncoproteins E6 and E7 bypass of critical cell-cycle checkpoints resulting in a prolonged and aberrant S-Phase induction cycle. During the transcriptional activation of the aberrant cell cycle, levels of MCM2 and TOP2A proteins increase in the proliferating cells.

Both the MCM2 and TOP2A proteins have been shown to be over-expressed in a number of different dysplastic and malignant tissues including cervical neoplasia^{1,6}. The over-expression of these proteins in morphologically abnormal cells, as demonstrated by a moderate-to-intense nuclear staining pattern using immunohistochemical (IHC) techniques, is indicative of the presence of aberrant S-Phase induction.

3. REAGENT PROVIDED

ProEx[™] C Antibody Reagent contains mouse monoclonal anti-MCM2 and anti-TOP2A purified from tissue culture supernatant and diluted in buffered saline solution containing protein stabilizers and 0.09% sodium azide.

4. PRINCIPLES OF PROCEDURE

Formalin-fixed paraffin-embedded tissue specimens are sectioned, deposited onto glass slides and deparaffinized. The sectioned specimens are pretreated with a buffer to expose antigenic sites. Blocking agents are added to minimize background staining caused by endogenous peroxidase or non-specific protein binding. The sample is then incubated with the ProEx[™] C Antibody Reagent. The addition of an enzyme-linked antibody chromogen system results in the formation of a visible chromogenic product localized at the antigen-antibody binding sites. The specimen is then counterstained with hematoxylin, a bluing agent is applied and the slide is coverslipped. Results are interpreted by a trained professional using a light microscope.

5. MATERIALS AND REAGENTS REQUIRED BUT NOT SUPPLIED (for Ventana Benchmark[®] XT Procedure)

- 10X Reaction Buffer – Cat # 950-300 2L (Ventana Medical Systems)
- LCS (Liquid Coverslip) – Cat # 650-010 2L (Ventana Medical Systems)
- 10X SSC – Cat # 950-110 2L (Ventana Medical Systems)
- EZ Prep – Cat # 950-102 2L (Ventana Medical Systems)
- CC1 (Cell Conditioning) – Cat # 950-124 2L (Ventana Medical Systems)
- iView[™] DAB Detection Kit – Cat # 760-091 (Ventana Medical Systems)
- Amplification Kit (A&B) – Cat # 780-285 (Ventana Medical Systems)
- Prep Kit Dispenser with Prep Kit Button – Cat # 786-3034 (Ventana Medical Systems)
- Hematoxylin – Cat # 760-2021 (Ventana Medical Systems)
- Bluing Reagent – Cat # 760-2037 (Ventana Medical Systems)
- Glass Slides
- Mounting Media (Acryto[®] or equivalent)
- Timer (capable of 1-60 minute intervals)
- Distilled H₂O
- Ethanol 95%, 100%

- Glass Coverslips
- Lab Marker
- 20L Carboy (Nalgene[®] or equivalent)
- Slide Dryer
- Slide Rack with Staining Dishes
- Xylene or Xylene Substitutes
- Light Microscope (10x, 20x [optional], 40x objectives)

6. PRECAUTIONS

- 6.1. For *in vitro* diagnostic use.
- 6.2. Slide clearing steps requiring xylene must be performed in a certified, chemical fume hood.
- 6.3. The ProEx[™] C Antibody Reagent contains sodium azide (NaN₃), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
- 6.4. DAB (3,3'-Diaminobenzidine) is classified as a suspected carcinogen. Avoid physical contact and prolonged or repeated exposure. Use in a certified, chemical fume hood.
- 6.5. Specimens and all materials exposed to specimens should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents come in contact with sensitive areas, wash with copious amounts of water.
- 6.6. Minimize microbial contamination of reagents to avoid nonspecific staining.
- 6.7. Incubation times, temperatures or methods other than those specified may give erroneous results.
- 6.8. Do not use the ProEx[™] C Antibody Reagent after the expiration date stamped on the package. The user must validate conditions if reagents are stored under any conditions other than those specified in the package insert.
- 6.9. Wear appropriate Personal Protective Equipment to avoid reagent contact with eyes and skin. Refer to the Material Safety Data Sheet (MSDS) for additional information.

7. INSTRUCTIONS FOR USE**7.1. Specimen Preparation**

- 7.1.1. Cut 4 μm sections from the tissue block and place the sections on SuperFrost Plus glass slides.
- 7.1.2. Label the slides.
- 7.1.3. Bake the slides in a forced air oven for 20 minutes. If the slides are already dry, touch the slide to a Histo-Orienter until the paraffin melts.

7.2. Reagent Preparation for the Ventana Benchmark[®] XT

Note: Refer to manufacturer's instructions.

- 7.2.1. 10X Reaction Buffer
 - 7.2.1.1. Register a new bottle of the 10X Reaction Buffer concentrate.
 - 7.2.1.2. Add 1 (one) bottle of 10X Reaction Buffer concentrate to 18 liters of distilled water. Mix well.
 - 7.2.1.3. Fill up the EZ Prep bottle #4 on the Benchmark[®] XT. Check to ensure that the pH is between 6.5 – 7.1.
- 7.2.2. EZ Prep (10X) – Deparaffinization Solution
 - 7.2.2.1. Register a new bottle of EZ Prep concentrate.
 - 7.2.2.2. Add 1 (one) bottle of EZ Prep concentrate to 18 liters of distilled water. Mix well.
 - 7.2.2.3. Fill up the EZ Prep bottle #1 on the Benchmark[®] XT. Check to ensure that the pH is between 6.90 – 7.2.
- 7.2.3. SSC (10X)
 - 7.2.3.1. Register 2 (two) bottles of SSC concentrate.
 - 7.2.3.2. Add 2 (two) bottles of SSC concentrate to 16 liters of distilled water. Mix well.
 - 7.2.3.3. Fill up the SSC bottle #3 on the Benchmark[®] XT. Check to ensure that the pH is between 7.1 – 7.5.
- 7.2.4. Fillable Prep Kit
 - 7.2.4.1. Register Prep Kit button
 - 7.2.4.2. Fill Prep Kit Dispenser according to manufacturer's instructions.

7.3. Staining Procedure Notes

- 7.3.1. This protocol is for use with automated staining on the Ventana Benchmark[®] XT using the iView[™] detection chemistry.
- 7.3.2. Do not allow the slides to dry out at any time during the procedure. Slides that have been allowed to dry out during the procedure may result in increased background staining.

8. AUTOMATED STAINING PROTOCOL (for the Ventana Benchmark® XT)

- 8.1. Turn on the staining module on the Ventana Benchmark® XT and start the software. Refer to the manufacturer's operating instructions for the Ventana Benchmark® XT.
- 8.2. Select the following protocol parameters on the Benchmark® XT software.
 - 8.2.1. Paraffin (selected)
 - 8.2.2. Deparaffinization (selected)
 - 8.2.3. Cell Conditioning (selected)
 - 8.2.4. Conditioner #1 (selected)
 - 8.2.5. Mild CC1 (selected)
 - 8.2.6. Standard CC1 (selected)
 - 8.2.7. Extended CC1 (selected)
 - 8.2.8. Ab Incubation Temperatures (selected)
 - 8.2.9. 37 C Ab. Inc. (selected)
 - 8.2.10. Antibody (selected)
 - 8.2.11. Apply 1 drop of [PREP KIT 100] (Antibody) and incubate for (1 hour).
 - 8.2.12. Amplify (selected)
 - 8.2.13. Counterstain (selected)
 - 8.2.14. Apply 1 drop of [Hematoxylin] (Counterstain), apply coverslip and incubate for (8 minutes).
 - 8.2.15. Post Counterstain (selected)
 - 8.2.16. Apply 1 drop of [Bluing Reagent] (Post Counterstain), apply coverslip and incubate for (4 minutes).
- 8.3. Register all reagents being used in the assay.
- 8.4. Determine the number of slides to be stained (including controls).
- 8.5. Select or create labels for each slide ensuring that each label corresponds to a single staining protocol.
- 8.6. Apply the appropriate label to each slide.
- 8.7. Load the slides onto the slide carousel.
- 8.8. Load the reagent dispensers and mount the reagent tray onto the reagent carousel.
- 8.9. Fill the bulk reagent carboys.
- 8.10. Check the waste module carboy and empty, if necessary.
- 8.11. Select "RUN" on the main computer screen of the Benchmark® XT to begin staining.
- 8.12. After the run is completed, print out the protocol summary and staining run reports.
- 8.13. Remove the slides from the instrument and rinse the slides in soapy water for 3-5 minutes or until Liquid Coverslip residue is no longer visible.
- 8.14. Dehydrate the slides.
 - 8.14.1. Immerse slides in 95% ethanol, 1 minute or 25 dips.
 - 8.14.2. Immerse slides in absolute alcohol, 4 changes, 1 minute each or 25 dips.
 - 8.14.3. Clear with xylene, 3 changes, 1 minute each or 25 dips.
- 8.15. Coverslip slides with non-aqueous, permanent mounting media using glass coverslips.

9. STABILITY

- 9.1. When stored at recommended temperatures, unopened reagent vials are stable until the expiration date indicated on the vial.
- 9.2. Once opened, reagents are stable for ninety (90) days when stored at recommended temperatures.

10. QUALITY CONTROL

- 10.1. Variability in results is often derived from differences in specimen handling which deviates from recommended test procedures. Consult the quality control guidelines of the College of American Pathologists (CAP) Certification Program for Immunohistochemistry for additional information.
- 10.2. A positive tissue control should be included with each stain run to verify the assay performance. If the positive tissue control does not exhibit positive staining, the results with the other test specimens should be considered suspect or invalid.
- 10.3. A negative tissue control should be included with each stain run to verify the specificity of the primary antibody and to provide an indication of background staining. If the negative tissue control exhibits positive specific staining, the results with the other test specimens should be considered suspect or invalid.
- 10.4. A non-specific negative control reagent may also be used in place of the primary antibody to evaluate non-specific or background staining.

11. INTERPRETATION

Moderate-to-intense brown staining in the nucleus of cells indicates the presence of aberrant S-Phase induction. A pathologist should evaluate the stained slides using a light microscope. Results interpretation must be made by a certified professional within the context of the patient's history and other diagnostic tests.

12. LIMITATIONS

- 12.1. Immunohistochemical staining requires specialized training in the selection and application of reagents.
- 12.2. This reagent will perform 50 tests assuming 100µL of reagent is applied per slide.
- 12.3. Some normal cells may stain positive for aberrant S-Phase induction.
- 12.4. Optimal tissue staining is dependent upon fixation and processing of the specimen.
- 12.5. Non-specific or increased background staining may occur due to, but not limited to, variations in procedure, inadequate rinsing between assay steps, and/or inadequately processed specimens.

13. TROUBLESHOOTING

Problem	Possible Cause	Action
No staining on positive control slides	Reagents applied in improper order.	Review staining protocol.
	Omission of any reagent.	Repeat staining protocol.
Weak staining on positive slides	Insufficient antigen retrieval.	Check incubation times and temperature of antigen/epitope retrieval buffer.
	Incorrect antigen retrieval buffer used.	Review staining protocol.
	Inadequate incubation of primary antibody.	Review staining protocol.
	Primary antibody has been diluted.	Use primary antibody according to manufacturer's directions.
Excessive background staining	Inadequate rinsing between assay steps.	Repeat staining protocol.
	Excessive incubation times with key reagents.	Review staining protocol.
	Slides drying out during post assay processing.	Repeat staining protocol.

14. REFERENCES

1. Kastan M and Bartec J. Cell cycle checkpoints and cancer. *Nature*, 2004. Vol32:316-323.
2. Massague J. G1 cell-cycle control and cancer. *Nature*, 2004. Vol 432:298-432.
3. Freeman A, Morris LS, Millis AD, Stoeber K, Laskey RA, Williams GH and Coleman N. Minichromosome Maintenance Proteins as Biological Markers of Dysplasia and Malignancy. *CI Cancer Research*, 1999. Vol 5:2121-2132.
4. Ishimi Y, Okayasu I, Kato C, Kwon H, Kimura H, Yamada K and Song S. Enhanced expression of MCM proteins in cancer cells derived from uterine cervix. *Eur. J. Biochem* 2003. 270:1089-1101.
5. Lei M and Tye BK. Initiating DNA synthesis: from recruiting to activating the MCM Complex. *J Cell Science* 2001. Vol. 114 (8): 1447-1454.

15. GLOSSARY OF SYMBOLS



Catalog number



For *in vitro* diagnostic use



Consult instructions for use



Contains 7 mL



Caution, consult accompanying document



Storage Temperature Limitations



Batch Code



Use by YYYY-MM-DD or YYYY-MM



Manufacturer

TECHNICAL INFORMATION

In the United States, telephone TriPath Technical Services, toll-free 1-866-874-7284.

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