TRIPATH IMAGING

# ProEx C
aberrant S-Phase induction

For HISTOLOGY

REF 005-11000-40 | Σ 7 mL | CE

IVD

2°C – 8°C

**REAGENT DESCRIPTION**

| | |
|---|---|
| **Clone** | **MCM2 26H6.19, MCM2 27C5.6, TOP2A SWT3D1** |
| **Ig Class** | **$IgG_1$** |
| **Immunogen** | **Recombinant Human MCM2 and TOP2A** |

**1. INTENDED USE**

For In Vitro Diagnostic use.

For use with manual staining or automated staining using the Dako Envision®+ 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[1-5]. 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. 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 and the slide is coverslipped. Results are interpreted by a trained professional using a light microscope.

ProEx™ C Immunohistochemical Test is applicable for both manual and automated staining.

**5. MATERIALS AND REAGENTS REQUIRED BUT NOT SUPPLIED (for Manual Procedure)**

- EDTA 5X – Cat #CB9172 (BioCare)
- EnVision®+ Kit – Cat # K4007 (Dako)
- Mayer's Hematoxylin – Cat # S3309 (Dako)
- Wash Buffer 10X – Cat # S3006 (Dako)
- Universal Mouse Negative Control – Cat # N1698 (Dako)
- Pressure Cooker
- Glass Slides
- Mounting Media (ShurMount® or equivalent)
- Pipettes and Pipette Tips (capable of delivering 20μL, 200μL and 1000μL volumes)
- Timer (capable of 1-60 minute intervals)
- Deionized Water
- Ethanol 95%, 100%
- Glass Coverslips
- Lab Marker
- 10L Carboy (Nalgene® or equivalent)
- Sterile Disposable Bottles
- 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.** 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.

**6.4.** The ProEx™ C Antibody Reagent contains sodium azide ($NaN_3$), 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.

**6.5.** 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.

**7.2. Reagent Preparation**

7.2.1. 1X Wash Buffer, 10L

7.2.1.1. Combine 9 liters of deionized $H_2O$ with 1 liter of 10X Wash Buffer.

7.2.1.2. Cap and mix by inverting several times.

7.2.1.3. Label with an expiration date of 5 days from the date of preparation.

7.2.1.4. Store at room temperature (20-25 °C).

7.2.2. 1X EDTA (prepare fresh daily)

7.2.2.1. Add 40 mL of 5X EDTA to a clean 250 mL beaker.

7.2.2.2. Add 160 mL deionized $H_2O$.

7.2.2.3. Mix by gently stirring.

7.2.3. Hematoxylin (prepare fresh daily)

7.2.3.1. Add 20 mL of distilled $H_2O$ to a 25 mL test tube.

7.2.3.2. Add 1 mL of Mayer's Hematoxylin.

7.2.3.3. Cap the test tube and mix by inverting several times.

**8. STAINING PROTOCOL (Manual or Automated)**

**8.1. Staining Procedure Notes**

8.1.1. This protocol can be used with manual staining or automated staining using the Dako Envision®+ detection chemistry.

8.1.2. All reagents should be equilibrated to room temperature (20-25°C) prior to immunostaining.

8.1.3. All incubations should be performed at room temperature unless noted.

8.1.4. Do not allow slides to dry out during the staining procedure. Dried preparations may display increased non-specific staining. Slides should be placed in a humid chamber for prolonged incubations.

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**8.2. Epitope Retrieval**

8.2.1. De-paraffinize slides in 3 changes of xylene (five minutes each) followed by 3 changes of 100% alcohol (five minutes each).

8.2.2. Rehydrate the slides by rinsing in deionized $H_2O$.

8.2.3. Submerge slides in prepared 1X EDTA buffer solution and place the container into a pressure cooker. Heat the slides in the pressure cooker for 30 seconds.

8.2.4. When the heating time point is complete, allow slides to remain in the buffer in the pressure cooker and cool down for 10 minutes.

8.2.5. Rinse the slides with deionized $H_2O$ and transfer to a clean coplin jar containing 1X Wash Buffer.

**8.3. Peroxidase Blocking Reagent**

8.3.1. Tap off excess Wash Buffer.

8.3.2. Load slides into a prepared humidity chamber (filled with water moistened paper towels or gauze).

8.3.3. Apply 200μL Peroxidase Block reagent to cover the tissue section.

8.3.4. Incubate 5 minutes ($\pm$1 minute).

8.3.5. Rinse slides in 1X Wash Buffer, 3 changes, 2 minutes each.

**8.4. Primary Antibody Reagent**

8.4.1. Tap off excess Wash Buffer.

8.4.2. Load the slides into the prepared humidity chamber (filled with water moistened paper towels or gauze).

8.4.3. Apply 200μL ProEx™ C Antibody Reagent to completely cover tissue section.

8.4.4. Incubate 40 minutes at room temperature (20-25°C).

8.4.5. Rinse each slide individually with Wash Buffer using a wash bottle (do not focus the flow directly on the tissue section).

8.4.6. Rinse slides in Wash Buffer, 3 changes, 2 minutes each.

**8.5. Detection Chemistry (using EnVision®+ Reagents)**

8.5.1. Tap off excess Wash Buffer.

8.5.2. Load slides into prepared humidity chamber (filled with water moistened paper towels or gauze).

8.5.3. Apply 200μL HRP-Polymer reagent to completely cover tissue section.

8.5.4. Incubate 30 minutes ($\pm$1 minute).

8.5.5. Rinse slides in Wash Buffer, 3 changes, 2 minutes each.

8.5.6. Tap off excess buffer.

8.5.7. Load slides into prepared humidity chamber (filled with water moistened paper towels or gauze).

8.5.8. Apply 200μL of DAB working solution Reagent to cover tissue section.

8.5.9. Incubate for 5 minutes ($\pm$1 minute).

8.5.10. Rinse slides in running distilled $H_2O$ for 5 minutes.

8.5.11. Rinse slides in Wash Buffer, 1 changes for 2 minutes.

**8.6. Counterstain**

8.6.1. Load slides into prepared humidity chamber (filled with water moistened paper towels or gauze).

8.6.2. Apply 200μL of Hematoxylin counterstain to completely cover tissue section.

8.6.3. Incubate for 5 minutes ($\pm$10 seconds).

8.6.4. Rinse slides for 3 minutes in running $H_2O$.

**8.7. Mounting**

8.7.1. Immerse slides in 95% ethanol, 1 minute or 25 dips.

8.7.2. Immerse slides in absolute alcohol, 4 changes, 1 minute each or 25 dips.

8.7.3. Clear with xylene, 3 changes, 1 minute each or 25 dips.

8.7.4. 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 25 tests assuming 200μL 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. |
| | Incorrect preparation of DAB. | Prepare according to manufacturer's specifications, and repeat staining protocol. |
| Weak staining on positive control slides | Insufficient antigen retrieval. | Check incubation times and temperature of antigen/epitope retrieval buffer (refer to section 8.2). |
| | Incorrect antigen retrieval buffer used. | Review staining protocol and prepare buffer according to manufacturer's directions. |
| | Inadequate incubation of primary antibody. | Review staining protocol and adjust incubation times accordingly. |
| | Primary antibody has been diluted. | Use primary antibody according to manufacturer's directions. |
| | Excessive wash buffer remaining on slide prior to application of next reagent. | Repeat staining protocol. Tap and wipe away excess wash buffer. |
| Excessive background staining | Inadequate rinsing between assay steps. | Repeat staining protocol. Add additional wash buffer rinse steps. |
| | Excessive incubation times with key reagents. | Review staining protocol and adjust incubation times accordingly. |
| | Slides drying out during the assay incubation or rinsing steps. | Repeat staining protocol. Check humidity chamber. |

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## 14. REFERENCES


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## 15. GLOSSARY OF SYMBOLS

| Symbol | Meaning |
|---|---|
| REF | Catalog number |
| IVD | For *in vitro* diagnostic use |
| | Consult instructions for use |
| Σ | Contains 7 mL |
| | Caution, consult accompanying document |
| | Storage Temperature Limitations |
| LOT | 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.

TRIPATH IMAGING

TriPath Imaging, Inc.
780 Plantation Drive
Burlington, NC 27215 USA
(800) 426-2176


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Millennium Pharmaceuticals, Inc.
40 Landsdowne Street
Cambridge, MA 02139 USA
www.millennium.com


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