TRIPATH IMAGING®

ProEx™ C

aberrant S-Phase induction

For CYTOLOGY

REF 005-11000-20 Σ 75 CE

IVD

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.

The ProEx™ C Immunocytochemical Test is intended for the qualitative evaluation of aberrant S-Phase induction in cervical cytology specimens. The test results provide adjunctive information for the cervical cytological interpretation of morphologically abnormal epithelial cells as defined by Bethesda 2001 criteria. 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, 2, 3]. The over-expression of these proteins in morphologically abnormal cells, as demonstrated by a moderate-to-intense nuclear staining pattern using immunocytochemical (ICC) or 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

Cervical cytology specimens are deposited onto glass slides and immersed in a pretreatment 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 a unique, enzyme-linked antibody chromogen system results in the formation of a visible colorimetric 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 using a light microscope.

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

5. MATERIALS AND REAGENTS REQUIRED BUT NOT SUPPLIED

- SureDetect™ Slide Preparation Buffer 10X – REF 090-11008-00 (TriPath Imaging®, Inc.)
  - Pretreatment Buffer
- SureDetect™ Detection Reagents – REF 005-11000-01 (TriPath Imaging®, Inc.)
  - Peroxidase Block
  - Protein Block
  - Mouse Probe
  - Polymer Reagent
  - DAB
  - DAB Substrate Buffer
- SureDetect™ Counterstains – REF 005-11000-02 (TriPath Imaging®, Inc.)
  - Mayer's Hematoxylin
  - Bluing Agent
- SureDetect™ SiHa Cell Control – REF 005-11012-00 (TriPath Imaging®, Inc.)
- Deionized or Distilled Water
- Absorbent Wipes
- Ethanol (95% and 100%)
- Glass Slides
- Glass Coverslips
- Gloves
- Humid Chamber
- Light Microscope [10x, 20x (optional), 40x objectives]
- Mounting Media
- Pipettes and Pipette Tips (capable of delivering 20µL, 200µL and 1000µL volumes)
- Staining Jars or Containers
- Timer (capable of 1-60 minute intervals)
- Tris Buffered Saline with 0.05% Tween 20 (TBST)
- Universal Mouse IgG Negative Control
- Vortexer
- Xylene or Xylene Substitutes
- Steamer/waterbath

6. PRECAUTIONS

6.1. For *In vitro* Diagnostic Use.

6.2. Cervical cytology slides must be placed into the SureDetect™ Slide Preparation Buffer as soon as they are prepared. The slides must remain in the SureDetect™ Slide Preparation Buffer for at least 1 hour, but not longer than 72 hours prior to staining.

6.3. 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.

6.4. This 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. The ProEx™ C Antibody Reagent has been titered for optimal performance. Further dilution may result in loss of antigen staining.

6.9. 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.

6.10. Wear appropriate Personal Protective Equipment to avoid reagent contact with eyes and skin. Refer to the Material Safety Data Sheet (MSDS) for additional information.

6.11. The ProEx™ C Antibody Reagent is intended for use with cervical cytology specimens that have been fixed with an ethanol based fixative. Compatibility with other cytology preparations has not been evaluated.

7. INSTRUCTIONS FOR USE

7.1. Specimen Preparation

7.1.1. Slides must be placed into the SureDetect™ Slide Preparation Buffer as soon as they are prepared (Refer to the SureDetect™ Slide Preparation Buffer 10X for directions on preparation of a working slide solution). The slides must remain in the buffer for at least 1 hour, but not longer than 72 hours prior to staining.

7.1.2. An epitope retrieval procedure must be used for optimal performance. This procedure involves soaking prepared slides in a working solution of SureDetect™ Slide Preparation Buffer for a minimum of 1 hour at room temperature followed by heating slides in the same buffer to 95°C. Slides are held at 95°C for 15 minutes and are allowed to cool at room temperature (20-25°C) for 20 minutes. The use of a calibrated waterbath or vegetable steamer capable of maintaining the required temperature is recommended. Laboratories located at higher elevations should determine the best method of maintaining the required temperature. The staining procedure should be initiated immediately following epitope retrieval and cool down. Deviations from the described procedure may adversely affect results.

7.2. Reagent Preparation

7.2.1. Tris Buffered Saline (TBS) with 0.05% Tween 20 (TBST)

7.2.1.1. Prepare TBS according to manufacturer's specifications.

7.2.1.2. If not already present in TBS, add Tween 20 to a final concentration of 0.05%.

7.2.2. DAB Chromogen-Substrate Working Solution

7.2.2.1. Prepare DAB Chromogen-Substrate working solution according to manufacturer's specifications.

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8. STAINING PROTOCOL (Performed At Room Temperature, 20-25°C)

8.1. Staining Procedural notes

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

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

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

8.2. Epitope Retrieval

8.2.1. Place prepared slides in a container containing a working solution of SureDetect™ Slide Preparation buffer for a minimum of 1 hour up to a maximum of 72 hours.

8.2.2. Incubate in a waterbath or steamer for 15 minutes at 95°C.

8.2.3. Remove the entire container with slides from the waterbath or steamer and allow slides to cool in the buffer for 20 minutes.

8.2.4. Rinse the slides with deionized H_2O and transfer to a clean container containing TBST.

8.3. Blocking Reagent

8.3.1. Tap off excess buffer.

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

8.3.3. Apply 4-5 drops (200µL) of Peroxidase Block reagent to cover the cell deposition area.

8.3.4. Incubate 5 minutes (±1 minute).

8.3.5. Rinse slides in TBST, 3 changes, 2 minutes each.

8.4. Protein Block

8.4.1. Tap off excess buffer.

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

8.4.3. Apply 4-5 drops (200µL) of Protein Block to completely cover cell deposition area.

8.4.4. Incubate 5 minutes (±1 minute).

8.4.5. Do not rinse.

8.5. Primary Antibody Cocktail

8.5.1. Tap off excess Protein Block.

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

8.5.3. Apply 4-5 drops (200µL) of ProEx™ C Antibody Reagent to completely cover cell deposition area.

8.5.4. Incubate 30 minutes at room temperature (20-25°C).

8.5.5. Rinse each slide individually with TBST using a wash bottle (do not focus the flow directly on the cell deposition area). Load slides into a slide rack.

8.5.6. Rinse slides in TBST, 3 changes, 2 minutes each.

8.6. Detection Chemistry (using SureDetect™ General Use Reagents)

8.6.1. Tap off excess buffer.

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

8.6.3. Apply 4-5 drops (200µL) of Mouse Probe Reagent to completely cover cell deposition area.

8.6.4. Incubate 20 minutes (±1 minute).

8.6.5. Rinse slides in TBST, 3 changes, 2 minutes each.

8.6.6. Tap off excess buffer.

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

8.6.8. Apply 4-5 drops (200µL) of Polymer Reagent to cover cell deposition area.

8.6.9. Incubate for 20 minutes (±1 minute).

8.6.10. Rinse slides in TBST bath, 3 changes, 2 minutes each.

8.6.11. Tap off excess buffer.

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

8.6.13. Apply 4-5 drops (200µL) of Substrate-Chromogen solution (DAB) completely cover cell deposition area.

8.6.14. Incubate for 5 minutes (±1 minute).

8.6.15. Rinse slides for 5 minutes in deionized H_2O.

8.7. Counterstain

8.7.1. Rinse slides in TBST, 1 change for 2 minutes.

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

8.7.3. Apply 4-5 drops (200µL) of Hematoxylin counterstain to completely cover cell deposition area.

8.7.4. Incubate for 1 minute (±10 seconds).

8.7.5. Rinse slides for 3 minutes in running H_2O.

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

8.7.7. Blue slides by applying 4-5 drops (200µL) of Bluing Agent for 1 minute (±10 seconds).

8.7.8. Repeat running water rinse for 1 minute.

8.8. Mounting

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

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

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

8.8.4. Coverslip slides with non-aqueous, permanent mounting media and 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 (90) ninety 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 proposed quality control guidelines of the *Clinical and Laboratory Standards Institute, Quality Assurance for Immunocytochemistry* for additional information.

10.2. SureDetect™ SiHa Cell Control is available as a positive control from TriPath Imaging®, Inc. Each vial contains a cervical cancer cell line, which is processed in a similar manner as the clinical specimens. A universal mouse IgG negative control can be used as a negative control. A positive and negative control slide should be included in each staining procedure. The staining results should be used as an indication of the validity of the staining run.

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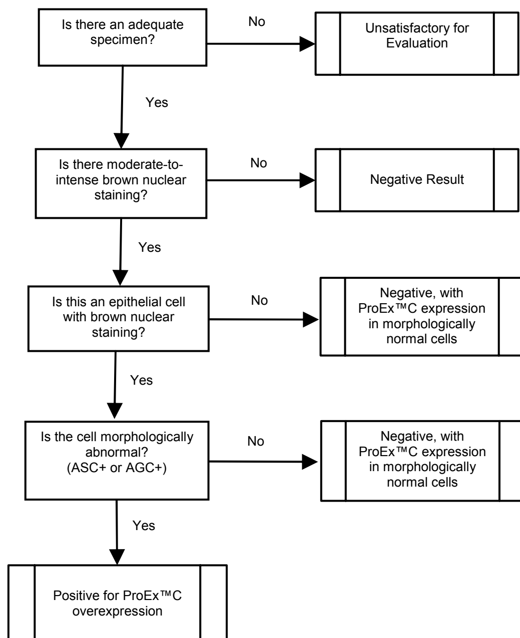
11. INTERPRETATION OF STAINING

11.1. Patient and Control Specimens: A cytotechnologist or 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.

11.2. Control Slides: The positive and negative stain control slides should be examined prior to the review of patient specimens to ascertain that all reagents functioned properly. The presence of a brown reaction product in the nuclei of the SiHa Cell Control slide stained with ProEx™ C is indicative of positive reactivity. The universal mouse IgG negative control slide stained with the same Detection Reagents should have no brown staining nuclei and should show staining only from the hematoxylin counterstain.

11.3. Scoring of Slides



12. LIMITATIONS

12.1. Immunocytochemical staining requires specialized training in the selection and application of reagents.

12.2. Some normal cells may stain positive for aberrant S-Phase induction.

12.3. 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.

12.4. This reagent will perform 75 tests assuming 200μL of reagent is applied per slide.

12.5. Age of specimen and handling of specimens other than manufacturer's recommended procedures can affect antigen-antibody site as well as staining quality.

13. TROUBLESHOOTING

Problem	Possible Cause	Action
No staining on positive control slides	Reagents applied in improper order.	Review staining protocol and repeat procedure.
	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 slides	Insufficient antigen retrieval.	Check incubation times and temperature of antigen/epitope retrieval buffer (refer to section 7.1.2).
	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 Protein Block remaining on slide prior to application of ProEx™ C antibody.	Repeat staining protocol. Tap and wipe away excess Protein Block.
	Excessive TBST remaining on slide prior to application of next reagent.	Repeat staining protocol. Tap and wipe away excess TBST.
Excessive background staining	Inadequate rinsing between assay steps.	Repeat staining protocol. Add additional TBST 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.
Loss of cells	Poor adherence of specimen to slide.	For pre-coated slides, check expiration date of slides. Contact slide manufacturer.

14. REFERENCES

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15. GLOSSARY OF SYMBOLS

REF — Catalog number

IVD — For *in vitro* diagnostic use

Consult instructions for use

Σ — Contains 75 slides

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.

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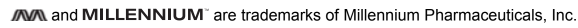

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