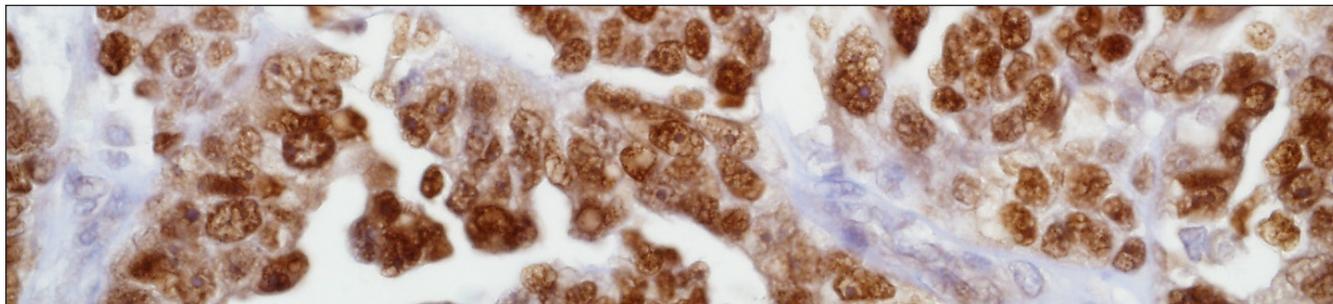


anti-PAX8 (MRQ-50)

Mouse Monoclonal Primary Antibody



PRODUCT AVAILABILITY

Ventana Cat. No.	Roche Cat. No.	Description
760-4618	06523927001	50 test dispenser

SYMBOL DEFINITIONS

A	ascites	E	serum
S	supernatant	KEY-CODE	keycode

INTENDED USE

This antibody is intended for *in vitro* diagnostic (IVD) use.

The Cell Marque PAX8 (MRQ-50) antibody is intended for qualified laboratories to qualitatively identify by light microscopy the presence of associated antigens in sections of formalin-fixed, paraffin-embedded tissue sections using IHC test methods. This antibody is used subsequent to a clinical diagnosis of malignancy as an aid to determine if the disease can be classified as renal cell carcinoma, thyroid carcinoma, and ovarian non-mucinous carcinoma within the context of an antibody panel, the patient's clinical history, and other diagnostic tests evaluated by a qualified pathologist.

SUMMARY AND EXPLANATION

This protein is a member of the paired box (PAX) family of transcription factors. Members of this gene family typically encode proteins which contain a paired box domain, an octapeptide, and a paired-type homeodomain. This nuclear protein is involved in thyroid follicular cell development and expression of thyroid-specific genes. Mutations in this gene have been associated with thyroid dysgenesis, thyroid follicular carcinomas, and atypical thyroid adenomas.

PAX-8 is expressed in the thyroid (and associated carcinomas), non-ciliated mucosal cells of the fallopian tubes, and simple ovarian inclusion cysts, but not normal ovarian surface epithelial cells. PAX-8 is expressed in a high percentage of ovarian serous, endometrioid,

and clear cell carcinomas, but only rarely in primary ovarian mucinous adenocarcinomas. Studies have also found PAX-8 expression in renal tubules as well as renal cell carcinoma, nephroblastoma, and seminoma. A study by Tong et al. showed that 98% of clear cell RCCs, 90% of papillary RCCs, and 95% of oncocytomas were positive for anti-PAX-8 with frequencies of positivity that are similar to anti-PAX-2. Therefore, anti-PAX-8 may be used as an additional immunohistochemical marker for renal epithelial tumors. Normal lung and lung carcinomas do not express PAX-8. Similarly, the absence of expression of PAX-8 in breast and other non-GYN carcinomas other than those primary to the thyroid indicates that anti-PAX-8 is an important new marker of ovarian cancer and a useful marker for the differential diagnoses in lung and neck tumors, or tumors at distant sites where primary lung carcinoma, breast carcinoma or thyroid carcinoma are possibilities. Anti-PAX-8, combined with organ system-specific markers such as anti-uroplakin III, anti-mammaglobin, and anti-TTF-1, can collectively serve as a very useful panel to determine the primary site of invasive micropapillary ovarian carcinomas from invasive carcinomas arising from bladder, lung, and breast.¹⁻⁹

PRINCIPLES AND PROCEDURES

Anti-PAX8 (MRQ-50) may be used as the primary antibody for immunohistochemical staining of formalin-fixed, paraffin-embedded tissue sections. In general, immunohistochemical staining in conjunction with a streptavidin-biotin detection system allows the visualization of antigens via the sequential application of a specific antibody (primary antibody) to the antigen, a secondary antibody (link antibody) to the primary antibody, an enzyme complex and a chromogenic substrate with interposed washing steps. Alternatively, a biotin-free detection system may be used. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. The specimen may then be counterstained and a coverslip applied. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

Anti-PAX8 (MRQ-50) is optimally diluted to be compatible with Ventana Roche detection kits and automated slide stainers. Each step in the staining protocol includes incubation for a precise time at a specific temperature. At the end of each incubation step, the sections are rinsed by the Ventana Roche automated slide stainer to stop the reaction and

remove unbound material that would hinder the desired reaction in subsequent steps. To minimize evaporation of the aqueous reagents from the specimen-containing slide, a coverslip solution is applied in the slide stainer. For more detailed information on instrument operation, refer to the appropriate Ventana Roche automated slide stainer Operator's Manual.

MATERIALS AND METHODS

Reagents Provided

One dispenser of PAX8 (MRQ-50) primary antibody contains sufficient prediluted reagent for 50 tests. The antibody is diluted in Tris Buffer, pH 7.3-7.7, with 1% BSA and <0.1% Sodium Azide.

The immunoglobulin concentration range for this product is 17.3-32.0 µg/ml.

The immunoglobulin concentration of the reagent appears on the product label.

Isotype: IgG

See product label for antibody source details.

Reconstitution, Mixing, Dilution, Titration

This antibody is optimized for use on a Ventana Roche automated slide stainer in combination with Ventana Roche detection systems. No reconstitution, mixing, dilution, or titration is required. Further dilution may result in loss of antigen staining. The user must validate any such changes. Differences in tissue processing and technical procedures in the laboratory may produce significant variability in results and require regular use of controls. (See Quality Control Procedures section)

Materials and Reagents Needed But Not Provided

The following reagents and materials may be required for staining but are not provided with the primary antibody:

- | | |
|---|---|
| 1. Positive and negative control tissue | BenchMark [®] ULTRA automated slide stainers |
| 2. Microscope slides, positively charged | 12. iVIEW [™] DAB (preferred), ultraView [™] , AEC, V Red (ALK PHOS) and Enhanced V Red detection kits |
| 3. Drying oven capable of maintaining a temperature of 58-60°C ± 5°C | |
| 4. Bar code labels (appropriate bar code labels for negative control and the primary antibody being tested) | 13. Detection system specific software (ES [®] automated slide stainer only) |
| 5. Staining jars or baths | 14. APK Wash Solution (ES [®] and NexES IHC [®] automated slide stainers) |
| 6. Timer | |
| 7. Amplifier (when applicable) | 15. Liquid Coverslip [™] solution (ES [®] and NexES IHC [®] automated slide stainers) |
| 8. Xylene or xylene substitute | |
| 9. Ethanol or reagent alcohol | 16. EZ Prep [™] solution (BenchMark [®] , BenchMark [®] XT, and BenchMark [®] ULTRA automated slide stainers) |
| 10. Deionized or distilled water | |
| 11. ES [®] , NexES IHC [®] , BenchMark [®] , BenchMark [®] XT, and | |

- | | |
|---|---------------------------------------|
| 17. Reaction Buffer (BenchMark [®] , BenchMark [®] XT, and BenchMark [®] ULTRA automated slide stainers) | 19. Hematoxylin or other counterstain |
| 18. LCS (BenchMark [®] , BenchMark [®] XT, and BenchMark [®] ULTRA automated slide stainers) | 20. Negative Control Reagent |
| | 21. Mounting medium |
| | 22. Cover glass |
| | 23. Light microscope (40-400x) |

Storage and Handling

Store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and stability of the antibody after every run, the cap must be replaced and the dispenser must be immediately placed in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date for the prescribed storage method.

There are no definitive signs to indicate instability of this product; therefore, positive and negative controls should be run simultaneously with unknown specimens. Contact Cell Marque customer service if there is a suspected indication of reagent instability.

Specimen Collection and Preparation for Analysis

Routinely processed, neutral-buffered formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with Ventana Roche detection systems and a Ventana Roche automated slide stainer (see Materials and Reagents Needed, But Not Provided section). The recommended tissue fixative is 10% neutral buffered formalin. Variable results may occur as a result of prolonged fixation or special processes such as decalcification of bone marrow preparations.

Each section should be cut to the appropriate thickness (approximately 3 µm) and placed on a positively charged glass slide. Slides containing the tissue section may be baked for at least 2 hours (but not longer than 24 hours) in a 58-60°C ± 5°C oven.

WARNINGS AND PRECAUTIONS

1. Take reasonable precautions when handling reagents. Use disposable gloves and lab coats when handling suspected carcinogens or toxic materials (example: xylene).
2. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
3. Patient specimens and all materials contacting them should be handled as biohazardous materials and disposed of with proper precautions. Never pipette by mouth.
4. Avoid microbial contamination of reagents, as this could produce incorrect results.
5. Incubation times and temperatures other than those specified may give erroneous results.
6. The reagents have been optimally diluted, and further dilution may result in loss of antigen staining. The user must validate any such

change.

7. When used according to instructions, this product is not classified as a hazardous substance. The preservative in the reagent is less than 0.1% sodium azide and does not meet the OSHA (USA) criteria for hazardous substance at the stated concentration. See MSDS.
8. The user must validate any storage conditions other than those specified in the package insert.
9. Diluent may contain bovine serum albumin and supernatant may contain bovine serum. The products containing fetal bovine serum and products containing bovine serum albumin are purchased from commercial suppliers. Certificates of Origin for the animal source used in these products are on file at Cell Marque. The certificates support that the bovine sources are from countries with negligible BSE risk and state sources of bovine from USA and Canada.
10. As with any product derived from biological sources, proper handling procedures should be used.

INSTRUCTIONS FOR USE

Step by Step Procedure

Cell Marque's primary antibodies have been developed for use on Ventana Roche automated slide stainers in combination with Ventana Roche detection kits and accessories.

Dispenser Preparation, Handling & Storage Instructions

Preparing For Use:

Where Used: For NexES® IHC, BenchMark® Series and Discovery® automated instruments, software version 8.0 and higher.

1. Shipping Key Removal

To remove the Shipping Key (shown in Figure A), remove the Nozzle Cap, hold the dispenser upright and pull the Key Tab to disengage it from each end. DO NOT cover the nozzle tip as it could permanently damage the dispenser. DO NOT depress the dispenser while removing the key as it could waste reagent. Discard the shipping key.

2. Preparing the Dispenser for Use

Remove the Nozzle Cap and place on the Nozzle Cap Holder. Fluid may be present inside the Nozzle Cap. Install the dispenser on the reagent carousel. The Inline Dispenser has been designed to be "Prepared for Use" by the NexES® software Version 8.0 or higher. Before each run, the software will detect a new dispenser on the carousel and prime it automatically. Manually priming the dispenser is not necessary and should NEVER be done as it could waste reagent and decrease the number of available dispenses.

Note - All earlier software installations: After removing the shipping key, remove the nozzle cap and CHARGE THE DISPENSER BY RAPIDLY PUMPING 3 to 4 TIMES, keeping the dispenser in an upright position. Charging is only necessary prior to first time use. (See Inspect Prime Before Use section.)

3. Dispenser Storage & Handling

To insure reliable operation, the dispenser must always be capped when not in use and should NEVER be manually dispensed. (See the Do's and Don'ts section.)

Do's and Don'ts

DO:

1. Check priming chamber and meniscus before each use. (See Inspect Prime Before Use below).
2. Store nozzle cap on dispenser. A holder is provided.
3. Cap dispenser when not in use to prevent evaporation. Dispensers mounted on the reagent tray can be capped (from underneath the tray) when not in use.
4. Store dispensers in an upright position in a rack and on the reagent carousel.
5. When mounting the dispenser on the carousel, grasp the coupler to avoid accidental manual dispensing.

DON'T:

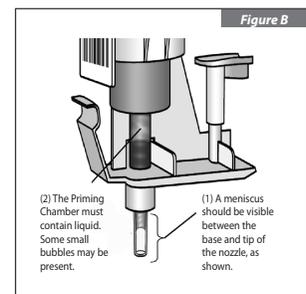
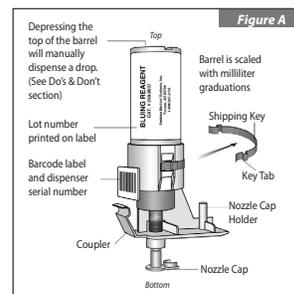
1. Do not manually dispense when inverted (upside down). Prime will be lost and may be impossible to restore.
2. Do not manually dispense with the nozzle cap in place. This can permanently damage the dispenser.
3. Do not manually dispense or prime prior to each use. This is not necessary and wastes reagent.
4. Do not hold the barrel in the down position. Fluid can leak from the dispenser when the barrel is depressed.
5. Do not stack carousels with dispensers installed. This can cause the dispensers to leak.

Inspect Prime Before Use:

Remove the nozzle cap and refer to Figure B.

Dispenser is ready for use when:

1. A meniscus is present in the area shown in Figure B.
2. The priming chamber contains liquid.



The procedures for staining on the Ventana Roche automated slide stainers are as follows. For more detailed instructions and additional protocol options refer to your Operator's Manual.

Recommended Staining Protocols for PAX8 (MRQ-50)

ultraView™:

1. Load slides, antibody, and ultraView™ detection kit dispensers onto

- BenchMark** instrument.
2. Select CC1 mild pretreatment.
 3. Antibody incubation should be set for 16 minutes at 37°C.
 4. Start the run.
 5. When the staining run is complete, move slides from instrument and rinse well with wash buffer.
 6. Coverslip.

OptiView:

1. Load slides, antibody, and detection kit dispensers onto BenchMark** instrument.
2. Select CC1 24 minute pretreatment.
3. Select pre primary peroxidase inhibitor.
4. Antibody incubation should be set for 8 minutes at 37°C.
5. Start the run.
6. When the staining run is complete, move slides from instrument and rinse well with wash buffer.
7. Coverslip.

QUALITY CONTROL PROCEDURES

Positive Tissue Control

A positive tissue control must be run with every staining procedure performed. This tissue may contain both positive and negative staining cells or tissue components and serve as both the positive and negative control tissue. Control tissues should be fresh autopsy, biopsy or surgical specimens prepared or fixed as soon as possible in a manner identical to the test sections. Use of a tissue section fixed or processed differently from the test specimen will serve to provide control for all reagents and method steps except fixation and tissue processing.

A tissue with weak positive staining is more suitable for optimal quality control and for detecting minor levels of reagent degradation. Positive tissue control for PAX8 (MRQ-50) primary antibody may include the following:

Ovarian carcinoma (non-mucinous carcinoma)	Nuclear
Thyroid carcinoma	Nuclear

Known positive tissue controls should be utilized only for monitoring the correct performance of processed tissues and test reagents, not as an aid in determining a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate appropriate positive staining, results with the test specimens must be considered invalid.

Negative Tissue Control

The same tissue used for the positive tissue control may be used as the negative tissue control. The variety of cell types present in most tissue sections offers internal negative control sites, but this should be verified by the user. The components that do not stain should demonstrate the absence of specific staining, and provide an indication of non-specific background staining. If specific staining occurs in the negative tissue control sites, results with the patient specimens must be considered invalid.

Unexplained Discrepancies

Unexplained discrepancies in controls should be referred to your local Ventana Roche office immediately. If quality control results do not meet specifications, patient results are invalid. See the Troubleshooting section of this insert. Identify and correct the problem, then repeat the entire procedure with the patient samples.

Negative Control Reagent

A negative control reagent must be run for every specimen to aid in the interpretation of results. A negative control reagent is used in place of the primary antibody to evaluate nonspecific staining. The slide should be treated with negative control reagent, matching the host species of the primary antibody, and ideally having the same IgG concentration. The incubation period for the negative control reagent should equal the primary antibody incubation period.

INTERPRETATION OF RESULTS

The immunostaining procedure run on Ventana Roche automated slide stainers causes a colored reaction product to precipitate at the antigen sites localized by the primary antibody. Refer to the appropriate detection system package insert for expected color reactions.

A qualified pathologist experienced in immunohistochemistry procedures must evaluate positive and negative tissue controls before interpreting results.

Positive Tissue Control

The stained positive tissue control should be examined first to ascertain that all reagents are functioning properly. The presence of an appropriately colored reaction product within the target cells is indicative of positive reactivity. Refer to the package insert of the detection system used for expected color reactions. Depending on the incubation length and potency of the hematoxylin used, counterstaining will result in a pale to dark blue coloration of cell nuclei. Excessive or incomplete counterstaining may compromise proper interpretation of results. If the positive tissue control fails to demonstrate appropriate positive staining, any results with the test specimens are considered invalid.

Negative Tissue Control

The negative tissue control should be examined after the positive tissue control to verify the specific labeling of the target antigen by the primary antibody. The absence of specific staining in the negative tissue control confirms the lack of antibody cross reactivity to cells or

cellular components. If specific staining occurs in the negative tissue control, results with the patient specimen are considered invalid. Nonspecific staining, if present, will have a diffuse appearance. Sporadic light staining of connective tissue may also be observed in sections from tissues that are not optimally fixed. Intact cells should be used for interpretation of staining results. Necrotic or degenerated cells show non-specific staining.

Patient Tissue

Patient specimens should be examined last. Positive staining intensity should be assessed within the context of any background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen in question was not detected, not that the antigen is absent in the cells or tissue assayed. A panel of antibodies may aid in the identification of false negative reactions (see Summary of Expected Results section). The morphology of each tissue sample should also be examined utilizing a hematoxylin and eosin stained section when interpreting any immunohistochemical result. The patient’s morphologic findings and pertinent clinical data must be interpreted by a qualified pathologist.

LIMITATIONS

1. This reagent is “for professional use only” as immunohistochemistry is a multiple step process that requires specialized training in the selection of the appropriate reagents, tissues, fixation, processing; preparation of the immunohistochemistry slide; and interpretation of the staining results.
2. For laboratory use only.
3. For *in vitro* diagnostic use.
4. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may result from variations in fixation and embedding methods, as well as from inherent irregularities within the tissue.
5. Excessive or incomplete counterstaining may compromise proper interpretation of results.
6. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology, other histopathological criteria as well as other diagnostic tests. This antibody is intended to be used in a panel of antibodies. It is the responsibility of a qualified pathologist to be familiar with the antibodies, reagents and methods used to produce the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
7. Cell Marque provides antibodies at optimal dilution for use as instructed. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test

procedures must accept responsibility for interpretation of patient results.

8. This product is not intended for use in flow cytometry; performance characteristics have not been determined.
9. Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated because of biological variability of antigen expression in neoplasms, or other pathological tissues. Contact Cell Marque customer service with documented unexpected reactions.
10. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.
11. When used in blocking steps, normal sera from the same animal source as the secondary antisera may cause false negative or false positive results because of the effect of autoantibodies or natural antibodies.
12. False positive results may be seen because of nonimmunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (example: liver, brain, breast, kidney) subject to the type of immunostaining technique used.
13. As with any immunohistochemistry test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells or tissue assayed.

Specific Limitations

1. The antibody is optimized for the incubation time specified in the Instructions for Use section in combination with Ventana Roche detection kits and the Ventana Roche automated slide stainers. Because of variation in tissue fixation and processing, it may be necessary to increase or decrease the primary antibody incubation time on individual specimens.
2. Cell Marque antibodies, when used in combination with Ventana Roche detection systems and accessories, detects antigen(s) that survive routine formalin fixation, tissue processing, and sectioning. Users who deviate from recommended test procedures are responsible for interpretation and validation of patient results.

Summary of Expected Results

See the following tables of reactivity:

Normal Study			
Tissue	# Stained	Total #	Notes
Brain	0	1	
Adrenal Cortex	0	1	
Ovary	1	1	Stromal cells +
Pancreas	1	1	Islet cells +, duct -, acini -
Parathyroid	1	1	

Normal Study			
Pituitary	0	1	
Testis	0	1	
Thyroid	1	1	Epithelial
Breast	1	1	
Spleen	1	1	Lymphoid +
Tonsil	1	1	Mature B-cells +
Thymus	0	1	Mature B-cells +
Bone Marrow	1	1	Mature B-cells +
Lung	1	1	
Heart	1	1	
Esophagus	0	1	
Stomach	0	1	
Small Intestine	0	1	
Colon	0	1	
Liver	1	1	
Salivary Gland	1	1	
Gall bladder	0	1	
Ureter	1	1	
Kidney	1	1	
Bladder	0	1	
Prostate	0	1	
Uterus	1	1	Endometrium +
Fallopian Tube	1	1	
Cervix	1	1	
Skeletal Muscle	0	1	
Smooth Muscle	1	1	
Skin	1	1	Epidermis +
Peripheral Nerve	0	1	
Mesothelium	0	1	
Fat	0	1	
Placenta	0	1	

This antibody stains small lymphoid cells as indicated in the published literature.

Disease Tissue Study			
Tissue	# Stained	Total #	Notes
Renal cell carcinoma	27	27	
Papillary thyroid carcinoma	6	6	
Colorectal carcinoma	0	28	
Lung adenocarcinoma	0	9	

Disease Tissue Study			
Breast carcinoma	0	5	
Pancreatic carcinoma	0	2	
Hepatocellular carcinoma	0	4	
Transitional cell carcinoma	3	4	
Melanoma	0	5	
Mesothelioma	0	9	
Gastrointestinal stromal tumor	0	2	

Also, it shows high sensitivity by revealing renal cell carcinoma, papillary thyroid carcinoma, and transitional cell carcinoma with high specificity for other tumors, such as colorectal carcinoma, lung adenocarcinoma, and breast carcinoma as indicated in the published literature.

TROUBLESHOOTING

1. If the positive control exhibits weaker staining than expected, other positive controls run during the same instrument run should be checked to determine if it is because of the primary antibody or one of the common secondary reagents.
2. If the positive control is negative, it should be checked to ensure that the slide has the proper bar code label. If the slide is labeled properly, other positive controls used on the same instrument run should be checked to determine if it is because of the primary antibody or one of the common secondary reagents. Tissues may have been improperly collected, fixed or deparaffinized. The proper procedure should be followed for collection, storage and fixation.
3. If excessive background staining occurs, high levels of endogenous biotin may be present. A biotin blocking step should be included unless a biotin-free detection system is being used in which case any biotin present would not be a contributing factor to background staining.
4. If all of the paraffin has not been removed, the deparaffinization procedure should be repeated.
5. If specific antibody staining is too intense, the run should be repeated with incubation time shortened by 4 minute intervals until the desired stain intensity is achieved.
6. If tissue sections wash off the slide, slides should be checked to ensure that they are positively charged.

For corrective action, refer to the Step By Step Procedure section, the automated slide stainer Operator's Manual, or contact Cell Marque customer service.

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www.cellmarque.com



EC REP

EMERGO EUROPE

Molenstraat 15, 2513 BH, The Hague, NL.

