# CINtec® *PLUS* Cytology Kit

REF 805-100

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IVD Σ 100

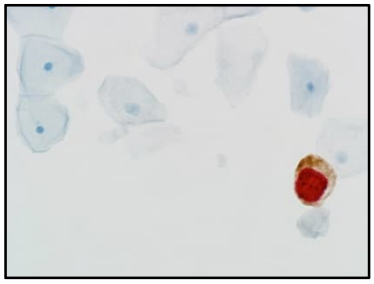

Figure 1. Cervical epithelial cell positive for p16$^{INK4a}$ (brown cytoplasmic stain) and for Ki-67 (red nuclear stain)

## INTENDED USE

For in vitro diagnostic (IVD) use.

CINtec® *PLUS* Cytology Kit is an immunocytochemistry assay for the simultaneous qualitative detection of the p16$^{INK4a}$ and Ki-67 proteins in cervical cytology preparations.

Interpretation of the test results may only be made by a certified professional in conjunction with the patient´s clinical history and additional diagnostic tests that have been performed.

## SUMMARY AND EXPLANATION

In eukaryotic cells, control of progression of the cell division cycle is regulated by a complex pattern of controlled expression and post-translational modifications of cell-cycle regulating proteins. The p16$^{INK4a}$ protein plays a major role in this mechanism of regulation of the eukaryotic cell cycle. It is part of the retinoblastoma protein (pRb)-mediated control of the G1/S phase transition, and it triggers cell cycle arrest in the course of cellular differentiation processes. Thus, p16$^{INK4a}$ provides an anti-proliferative effect during regular cell cycle progression. In terminally differentiated epithelial cells, p16$^{INK4a}$ expression is down-regulated to levels typically not detectable by immunocytochemistry[1].

Ki-67 is a proliferation-associated protein which can be detected exclusively in the nucleus of proliferating cells. Detailed cell cycle analyses have revealed that the Ki-67 antigen is present at detectable levels in all phases of cell proliferation as well as in mitosis, whereas quiescent or resting cells in the G0 phase do not express the antigen[2].

As cells over-expressing p16$^{INK4a}$ may actively proliferate only when the cell-cycle control mechanism is impaired, the expression of both p16$^{INK4a}$ and the proliferation marker Ki-67 within the same cell should mutually exclude each other under normal physiological conditions. Thus, concomitant expression of p16$^{INK4a}$ and Ki-67 in particular cells may be used as an indicator for the deregulation of the cell cycle control in the respective cells.

## PRINCIPLE OF THE PROCEDURE

The CINtec *PLUS* Cytology Kit contains a set of reagents for the simultaneous immunocytochemical detection of the p16$^{INK4a}$ and Ki-67 proteins in cytological specimens obtained from the uterine cervix. The proteins are detected using a ready-to-use cocktail of primary monoclonal antibodies which contains a monoclonal mouse antibody directed against human p16$^{INK4a}$ protein (clone E6H4™) and a primary recombinant rabbit antibody directed against human Ki-67 protein (clone 274-11 AC3). Following cell conditioning, inhibition of endogenous peroxidase activity and incubation with the primary antibody cocktail, the assay uses two ready-to-use detection systems optimized for use on cervical cytology specimens:

- A goat anti-mouse secondary antibody covalently attached to HQ haptens (proprietary hapten) and an anti-HQ hapten, horseradish peroxidase (HRP)-conjugated tertiary antibody optimized for the detection of the monoclonal mouse antibody clone E6H4;
- A goat anti-rabbit secondary antibody covalently attached to NP haptens (proprietary hapten) and an anti-NP hapten, alkaline-phosphatase (AP)-conjugated tertiary antibody optimized for the detection of the rabbit recombinant antibody clone 274-11 AC3.

The chromogenic reactions are based on the HRP-mediated conversion of 3,3'-diaminobenzidine tetrahydrochloride (DAB) and the AP-mediated conversion of Fast Red with Naphthol Phosphate resulting in a brown precipitate at the p16$^{INK4a}$ antigen site and a red precipitate at the Ki-67 antigen site, respectively.

After automated counterstaining and bluing, a two-step mounting procedure is followed. First, the slide is mounted using an aqueous mounting medium. Subsequently, the slide is coverslipped using a permanent mounting medium. The staining results are evaluated by light microscopy inspection.

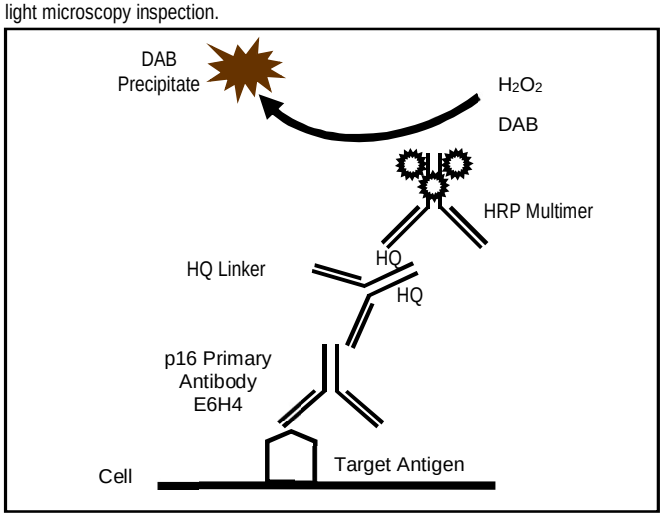


Figure 2. Detection of human p16$^{INK4a}$ protein

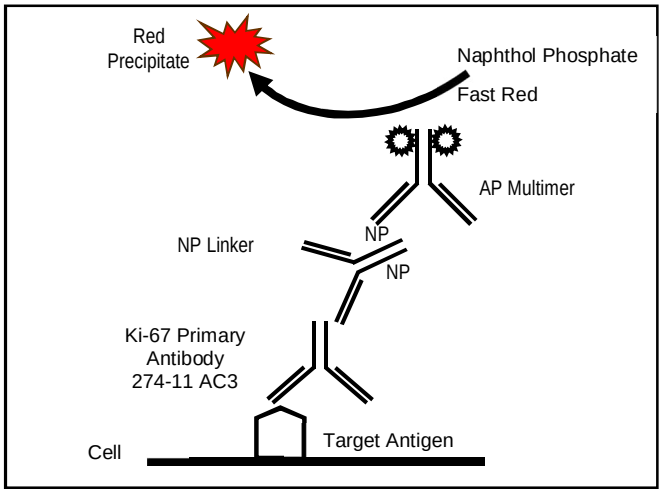


Figure 3. Detection of human Ki-67 protein

## MATERIAL PROVIDED

The CINtec *PLUS* Cytology Kit contains sufficient reagent for 100 tests.

| | |
|---|---|
| One 10 mL dispenser | CINtec PLUS Primary Antibody Cocktail (p16/Ki-67) contains a cocktail of monoclonal mouse antibody clone E6H4 directed to human p16$^{INK4a}$ protein and primary recombinant rabbit antibody clone 274-11 AC3 directed against human Ki-67 protein (<5 µg/mL total antibody) in a buffer containing protein with ProClin® 300, a preservative. |
| One 10 mL dispenser | CINtec *PLUS* Red anti-Rabbit NP Linker contains NP-labeled goat anti-rabbit IgG (<10 µg/mL; NP is a proprietary hapten covalently attached to the goat antibody) in a buffer containing protein with ProClin® 300, a preservative. |
| One 10 mL dispenser | CINtec *PLUS* Red AP Multimer contains a mouse monoclonal anti-NP-labeled AP tertiary antibody |

| | |
|---|---|
| | (<20 µg/mL) in a buffer containing protein with ProClin® 300, a preservative. |
| One 10 mL dispenser | CINtec *PLUS* Red Naphthol Phosphate contains Naphthol Phosphate (<1%) with ProClin® 300, a preservative. |
| One 10 mL dispenser | CINtec *PLUS* Fast Red contains Fast Red (<1%) in acetate buffer with ProClin® 300, a preservative. |
| One 10 mL dispenser | CINtec *PLUS* DAB Peroxidase Inhibitor contains hydrogen peroxide solution (<5%). |
| One 10 mL dispenser | CINtec *PLUS* DAB anti-Mouse HQ Linker contains HQ-labeled goat anti-mouse IgG (<40 µg/mL; HQ is a proprietary hapten covalently attached to the goat antibody) in a buffer containing protein with ProClin® 300, a preservative. |
| One 10 mL dispenser | CINtec *PLUS* DAB HRP Multimer contains a mouse monoclonal anti-HQ-labeled HRP tertiary antibody (<10 µg/mL) in a buffer containing protein with ProClin® 300, a preservative. |
| One 10 mL dispenser | CINtec *PLUS* DAB contains 3,3'-diaminobenzidine tetrahydrochloride (<1%) in a proprietary stabilizer solution with a proprietary preservative. |
| One 10 mL dispenser | CINtec *PLUS* DAB H2O2 contains hydrogen peroxide (<1%) in a phosphate buffer solution. |

## RECONSTITUTION, MIXING, DILUTION, TITRATION

The CINtec *PLUS* Cytology Kit is optimized for use on VENTANA BenchMark GX, XT and ULTRA automated slide stainers. No reconstitution, mixing, dilution, or titration of kit reagents is required.

Deviations from the recommended procedures for fixation and further processing of the cervical cytological specimens may produce substantial variability in results, necessitating regular performance of in-house controls.

For more information about controls, see the Quality Control section.

## MATERIALS REQUIRED BUT NOT PROVIDED

The following reagents and materials are required for staining but not provided with the CINtec *PLUS* Cytology Kit.

Not all products listed in the package insert may be available in all geographies. Consult your local support representative.

1. Appropriate controls (optional, please refer to Quality Control section)
2. Hematoxylin Counterstain
3. Bluing Reagent
4. Reaction Buffer Concentrate (10X)
5. Cell Conditioning Solutions (CC1/ ULTRA CC1)
6. Cell Conditioning Solutions (CC2/ ULTRA CC2), required on instrument but not used
7. EZ Prep Concentrate (10X), required on instrument but not used
8. SSC Concentrate (10X), required on instrument but not used
9. Liquid Coverslip (LCS/ ULTRA LCS)
10. Reagent Grade Ethanol denatured (purity ≥ 95%)
11. VENTANA BenchMark GX, XT or ULTRA automated slide stainer
12. SuperFrost® PLUS microscope slides (Thermo Fisher Scientific) for conventional smears are recommended
13. ThinPrep® Arcless Microscope Slides or ThinPrep® Microscope Slides FOR SPECIAL PROCESSING (Hologic Order # 70126-002)
14. BD SurePath™ PreCoat slides (included in the SurePath GYN kit)
15. CC/Mount™ aqueous mounting medium (Diagnostic BioSystems P/N: K 002; Sigma-Aldrich P/N: C9368)
16. Optional: Drying oven capable of maintaining a temperature of 60°C ± 5°C
17. Xylene (Histological grade)
18. Deionized or distilled water
19. Glass coverslips and Xylene-based mounting medium or plastic film coverslip method sufficient to cover cytology preparations
20. Light microscope
21. Mild dishwashing detergent

## STORAGE

Store at 2-8°C. Do not freeze. This reagent kit can be used immediately after removal from the refrigerator.

To ensure proper reagent delivery and the stability of the reagents, replace the dispenser's cap after every use and immediately place the dispensers in the refrigerator in an upright position.

The CINtec *PLUS* Cytology Kit has an expiration date. When properly stored, the reagents are stable through the date indicated on the label. Do not use the product beyond the expiration date for the prescribed storage method. There are no definitive signs to indicate instability of this product; therefore, a positive control should be run simultaneously with unknown specimens. Your local support representative should be contacted immediately if there is an indication of reagent instability.

## WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. Do not use the product if the packaging of any of its components is damaged. Should packaging be compromised or components damaged, please notify your local support representative without delay.
4. The reagents have been optimally diluted and further dilution may result in loss of antigen staining. The user must validate any such change.
5. ProClin® 300 is used as a preservative. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves. See the Material Provided section for a list of dispensers containing this preservative.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
7. Take reasonable precautions when handling reagents. Avoid contact of reagents with eyes, skin, and mucous membranes. Avoid inhalation of reagents. Use disposable gloves and wear suitable protective clothing when handling suspected carcinogens or toxic materials.
8. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. When handling and disposing of cytological specimens, including all specimens before and after fixation, as well as all materials exposed to them, adhere to the safety precautions for handling potentially infectious material as well as applicable waste disposal requirements.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

| Hazard | Code | Statement |
|---|---|---|
| Danger  | H317 | May cause an allergic skin reaction. |
| | H350 | May cause cancer. |
| | H412 | Harmful to aquatic life with long lasting effects. |
| | P201 | Obtain special instructions before use. |
| | P261 | Avoid breathing mist or vapours. |
| | P273 | Avoid release to the environment. |
| | P280 | Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. |
| | P308 + P313 | IF exposed or concerned: Get medical advice/ attention. |

| Hazard | Code | Statement |
|---|---|---|
| | P333 + P313 | If skin irritation or rash occurs: Get medical advice/ attention. |

## SPECIMEN PREPARATION

Cytological specimens must be adequately handled to preserve the specimens for immunocytochemical procedures. All specimens should be subjected to standard methods of cell processing.

To avoid obscuring elements such as blood and mucus and ensure a sample that is adequate per Bethesda Guidelines[3], clinicians should follow the recommended sampling techniques.[4]

The following slide preparation methods are suitable for use with the CINtec *PLUS* Cytology Kit:

- ThinPrep® (Hologic Inc.) slides prepared on a ThinPrep® 2000 or 5000 Processor (Hologic Inc.) using ThinPrep® Arcless Microscope Slides or ThinPrep® Microscope Slides FOR SPECIAL PROCESSING (Hologic Order # 70126-002) according to the manufacturer's recommendation;
- BD SurePath™ (BD Diagnostics Tripath) slides prepared according to the manufacturer´s recommendation;
- Manually prepared slides (conventional smear slides).

It is recommended to run appropriate controls simultaneously with patient specimens (please refer to Quality Control section for further details).

### ThinPrep® Sample Preparation

Cytologic sample in PreservCyt® Solution intended for immunocytochemistry staining using the CINtec *PLUS* Cytology Kit can be stored between 4°C and 30°C and tested within 6 weeks of collection.

ThinPrep® Arcless Microscope Slides or ThinPrep® Microscope Slides FOR SPECIAL PROCESSING (Hologic Order # 70126-002) are required for immunocytochemical staining with the CINtec *PLUS* Cytology Kit on VENTANA Benchmark GX, XT and ULTRA automated slide stainers.

#### ThinPrep® Sample Preparation using the ThinPrep® 2000 Processor

ThinPrep® (Hologic Inc.) slides are prepared on a ThinPrep® 2000 Processor (Hologic Inc.) according to the manufacturer's instructions. After the ThinPrep® 2000 Processor sequence is finished the processed slide sits in a fixative vial containing a ≥ 95% reagent grade ethanol solution. Using care, remove the vial from the fixative bath holder of the ThinPrep® 2000 Processor and transfer the processed slide from the vial into a slide bucket containing a ≥ 95% reagent grade ethanol solution. Incubate the slide in ethanol for a minimum of 15 minutes to a maximum of 60 minutes. Change the ethanol solution in the fixative vial and slide bucket after every 20 slides prepared. Upon completion of the incubation time remove the slide from the ethanol bucket and let dry lying horizontally on a flat surface for at least 60 minutes. Dried slides can be stored at room temperature protected from light and must be stained with the CINtec *PLUS* Cytology Kit within 3 days of preparation.

#### ThinPrep® Sample Preparation using the ThinPrep® 5000 Processor

ThinPrep® (Hologic Inc.) slides are prepared on a ThinPrep® 5000 Processor (Hologic Inc.) according to the manufacturer's instructions. After the ThinPrep® 5000 Processor sequence is finished the processed slides sit in a slide rack which is immersed in a ≥ 95% reagent grade ethanol solution containing fixative bath. Carefully remove the fixative bath from the ThinPrep® 5000 Processor and incubate slides for an additional minimum of 15 minutes to a maximum of 60 minutes. Change the ethanol solution in the fixative slide bucket after each run. Upon completion of the incubation time, remove the slides from the ethanol bucket and let dry lying horizontally on a flat surface for at least 60 minutes. Dried slides can be stored at room temperature protected from light and must be stained with the CINtec *PLUS* Cytology Kit within 3 days of preparation.

Before immunocytochemical staining with the CINtec *PLUS* Cytology Kit remove the original slide label used by the ThinPrep® 5000 Processor and apply the corresponding label generated by the VENTANA BenchMark GX, XT or BenchMark ULTRA automated slide stainer.

### BD SurePath™ Sample Preparation

Cytologic sample in SurePath™ Preservative Fluid intended for immunocytochemistry staining using the CINtec *PLUS* Cytology Kit can be stored for up to 4 weeks at room temperature (between 15°C and 30°C), or for 6 months in a refrigerator between 2°C and 10°C.

#### BD SurePath™ Sample Preparation Immediately Following Processing of a Pap Slide

Once an enriched cell pellet has been created for preparation of a slide for Pap staining, it can be used immediately for preparation of a second slide to be stained with the CINtec *PLUS* Cytology Kit. Follow manufacturer´s recommendation for use of the "Slide Preparation" [option 2] for GYN samples on the PrepStain™ instrument. The resuspension volume must be changed to 0 mL in the "Change Sample/Stain Parameters" menu option.

#### BD SurePath™ Sample Preparation from a Preserved Cell Pellet

Enriched cell pellets can be preserved by adding approximately 2 mL of SurePath™ Preservative Fluid and capping the sample tubes for storage (refer to manufacturer´s instructions for details). From the sample collection date, cell pellets that have been re-suspended in preservative fluid can be stored for up to 4 weeks at room temperature (between 15°C and 30°C), or for 6 months in a refrigerator maintained at 2°C to 10°C. To process a slide for the CINtec *PLUS* Cytology Kit, first bring the sample to room temperature for 60 minutes. Start with the second centrifugation step of the GYN enrichment process and proceed through the remainder of the pre-processing steps as outlined in the manufacturer´s instruction for reprocessing preserved cell pellets. Follow manufacturer´s recommendation for use of the "Slide Preparation" [option 2] for GYN samples on the PrepStain™ instrument.

For all preparation options listed above, remove the slide rack from the PrepStain™ instrument once the sample transfer step has been completed. Invert the rack to decant the liquid. Pipette 2 mL of a ≥ 95% reagent grade ethanol solution to each settling chamber and decant immediately. Rinse with 2 mL of a ≥ 95% reagent grade ethanol solution a second time and incubate for 10 minutes. Decant a second time by inverting the rack. Remove the settling chambers from the slides and let the slides dry lying horizontally on a flat surface for at least 60 minutes. Dried slides can be stored at room temperature protected from light and must be stained with the CINtec *PLUS* Cytology Kit within 3 days of preparation.

### Conventional Smear Preparation

Conventional smears should be fixed with cytological spray fixation reagent containing polyethylene glycol (e.g. Safetex™ Cytology Fixative, Andwin Scientific) immediately after sample collection. Spray-fixed conventional smear slides can be stored at room temperature protected from light and must be stained with the CINtec *PLUS* Cytology Kit within 7 days of preparation.

No further pre-processing is required prior to loading slides onto the VENTANA BenchMark GX, XT and ULTRA automated slide stainers.

## STAINING PROCEDURE

The CINtec *PLUS* Cytology Kit has been developed for use on VENTANA BenchMark GX, XT and ULTRA automated slide stainers in combination with VENTANA ancillary reagents and accessories. Refer to Table 2, Table 3, and Table 4 for recommended staining protocols.

The CINtec *PLUS* Cytology Kit has been optimized with the parameters indicated in Table 2, Table 3, and Table 4; nonetheless the user must validate results obtained with this kit.

The parameters for the automated protocols can be displayed, printed and edited according to the instructions in the Operator's Manuals for each instrument.

Table 2. Recommended Staining Protocol for ThinPrep®, SurePath™ and conventional smear slides on the VENTANA BenchMark GX automated slide stainer using Hematoxylin (P/N 760-2021) and Bluing Reagent (P/N 760-2037).

| Staining Procedure | GX CINtec *PLUS* Cytology | | |
|---|---|---|---|
| Selectable Options | Sample Preparation Type | | |
| | ThinPrep | SurePath | Conventional |
| ThinPrep | Selected | Not Selected | Not Selected |
| SurePath | Not Selected | Selected | Not Selected |
| Other | Not Selected | Not Selected | Selected |
| Cell Conditioning Option | Not Selectable | Not Selectable | 16 min |

| Staining Procedure | GX CINtec *PLUS* Cytology | | |
|---|---|---|---|
| Selectable Options | Sample Preparation Type | | |
| | ThinPrep | SurePath | Conventional |
| Antibody Inc Time | 16 min | 20 min | 16 min |
| HQ Linker Inc Time | 12 min | 16 min | 12 min |
| HRP Multimer Inc Time | 6 min | 8 min | 8 min |
| NP Linker Inc Time | 8 min | 16 min | 8 min |
| AP Multimer Inc Time | 8 min | 8 min | 8 min |
| Counterstain | Hematoxylin 4 min | Hematoxylin 4 min | Hematoxylin 4 min |
| Counterstain | Bluing 4 min | Bluing 4 min | Bluing 4 min |

Table 3. Recommended Staining Protocol for ThinPrep®, SurePath™ and conventional smear slides on the VENTANA BenchMark XT automated slide stainer using Hematoxylin (P/N 760-2021) and Bluing Reagent (P/N 760-2037).

| Staining Procedure | XT CINtec *PLUS* Cytology | | |
|---|---|---|---|
| Selectable Options | Sample Preparation Type | | |
| | ThinPrep | SurePath | Conventional |
| ThinPrep | Selected | Not Selected | Not Selected |
| SurePath | Not Selected | Selected | Not Selected |
| Other | Not Selected | Not Selected | Selected |
| Cell Conditioning Option | Not Selectable | Not Selectable | 16 min |
| Antibody Inc Time | 16 min | 20 min | 16 min |
| HQ Linker Inc Time | 12 min | 16 min | 12 min |
| HRP Multimer Inc Time | 8 min | 8 min | 8 min |
| NP Linker Inc Time | 8 min | 16 min | 8 min |
| AP Multimer Inc Time | 8 min | 8 min | 8 min |
| Counterstain | Hematoxylin 4 min | Hematoxylin 4 min | Hematoxylin 4 min |
| Counterstain | Bluing 4 min | Bluing 4 min | Bluing 4 min |

Table 4. Recommended Staining Protocol for ThinPrep®, SurePath™ and conventional smear slides on the VENTANA BenchMark ULTRA automated slide stainer using Hematoxylin (P/N 760-2021) and Bluing Reagent (P/N 760-2037).

| Staining Procedure | U CINtec *PLUS* Cytology | | |
|---|---|---|---|
| Selectable Options | Sample Preparation Type | | |
| | ThinPrep | SurePath | Conventional |
| ThinPrep | Selected | Not Selected | Not Selected |
| SurePath | Not Selected | Selected | Not Selected |
| Other | Not Selected | Not Selected | Selected |
| Cell Conditioning Option | Not Selectable | Not Selectable | 16 min |
| Antibody Inc Time | 16 min | 16 min | 16 min |
| HQ Linker Inc Time | 12 min | 16 min | 12 min |
| HRP Multimer Inc Time | 8 min | 8 min | 8 min |
| NP Linker Inc Time | 8 min | 16 min | 8 min |
| AP Multimer Inc Time | 8 min | 8 min | 8 min |
| Counterstain | Hematoxylin 4 min | Hematoxylin 4 min | Hematoxylin 4 min |
| Counterstain | Bluing 4 min | Bluing 4 min | Bluing 4 min |

### BenchMark GX, XT and ULTRA Automated Slide Stainer Operation

1. Apply slide bar code label corresponding to the protocol to be performed.
2. Load the CINtec *PLUS* Cytology Kit dispensers and required ancillary reagents onto the reagent tray and place them on the automated slide stainer.
3. Check bulk fluids and empty waste.
4. Load the slides onto the automated slide stainer.
5. Start the staining run.
6. At the completion of the run, remove the slides from the automated slide stainer.

### POST PROCESSING PROCEDURE - MOUNTING AND COVERSLIPPING

To maintain optimal sensitivity and to prevent fading of chromogens, a two-step mounting procedure is required.

Remove the slides from the VENTANA BenchMark GX, XT or ULTRA automated slide stainer and gently agitate and rinse slides with tap, deionized or distilled water and mild dishwashing detergent until the Liquid Coverslip is completely removed from the slides.

NOTE: Take care not to let the water directly hit the slides. Run the water at a minimal force.

The slides will be mounted following a two-step protocol and the following steps must be performed sequentially:

1. Aqueous mounting:
   - Incubate slides in distilled or deionized water for at least 1 min;
   - Slides not being coverslipped should remain in distilled or deionized water during application of the CC/Mount™ aqueous mounting media to the other slides;
   - Remove single slide from distilled or deionized water and carefully wipe the back side of the slide with a paper towel to remove excess water. Do not drain or wipe water off front of slide (sample side);
   - Hold slide at a slight angle and apply 4-6 drops of CC/Mount™ aqueous mounting medium (Diagnostic BioSystems P/N: K 002; Sigma-Aldrich P/N: C9368) per ThinPrep® or SurePath™ slide, and 8 drops per conventional smear slide. Avoid generation of air bubbles. To prevent bubble formation the first drop can be discarded onto a paper towel before applying CC/Mount™ on the specimen preparation area of the slide;
   - Gently tilt and rotate the glass slide to generate a thin layer of mounting medium to fully cover the specimen preparation area (do not yet apply a glass or film coverslip); check the distribution of the mounting medium on the slide by visual inspection;
   - Clean excess CC/Mount™ aqueous mounting media from the back and edges of the slide. Use wet paper towel if necessary;
   - For drying, place slides in a horizontal position:
     a) Incubate ThinPrep® or SurePath™ slides at 37-60°C for 1 hour, or alternatively overnight at room temperature;
     b) Incubate conventional smear slides at 37°C for 4 hours, or at 60°C for 1 hour, or alternatively overnight at room temperature;
2. Glass or film coverslipping:
   - After complete drying of the CC/Mount™ aqueous mount, allow slides to equilibrate to room temperature, if needed. Incubate slides in xylene for a minimum of 1 minute and up to a maximum of 20 minutes. Then, coverslip the

slides with glass coverslips using a xylene-based mounting medium or xylene based film coverslipping method.

NOTE: Slides must not be dehydrated by ascending series of alcohol before being glass or film coverslipped.

- Let the xylene-based mounting medium dry at room-temperature.

NOTE: To minimize fading, protect slides from light and store at room temperature.

## QUALITY CONTROL PROCEDURE

Deviations from the recommended procedures for fixation and further processing of the cervical cytological specimens may produce substantial variability in results. Malfunction of the product due to handling problems or to instability does not result in obvious signs. Therefore, appropriate controls should be run simultaneously with patient specimens.

### Positive Control

Specimens processed in the same manner as the patient sample(s) should be used as positive controls. Positive controls are indicative of correctly prepared specimens and proper staining techniques. One positive control should be included in each staining run.

Known positive controls should only be utilized for monitoring the correct performance of processed specimens and test reagents rather than as an aid in formulating a specific diagnosis of patient samples. If the positive controls fail to demonstrate appropriate positive staining, results with the test specimens should be considered invalid.

### Negative Control

A variety of different cell types present in representative cervical cytology specimens and that are known to be negative for the expression of the $p16^{INK4a}$ and Ki-67 antigens (such as superficial cells) may serve as an internal negative control to assess background staining.

### Assay Verification

The user should verify the performance of the CINtec PLUS Cytology Kit on positive and negative specimens with known performance characteristics prior to its initial use in a diagnostic procedure.

## STAINING INTERPRETATION / EXPECTED RESULTS

The CINtec *PLUS* Cytology Kit staining produces two distinct colored reaction products: a brown precipitate at the $p16^{INK4a}$ antigen sites, and a red precipitate at the Ki-67 antigen sites. Brown staining of cells (cytoplasm and/or nuclei) indicates $p16^{INK4a}$ over-expression. Red staining of cells (nuclei) indicates expression of Ki-67. Cells stained for both antigens exhibit brown cytoplasmic staining with typically pronounced red nuclei. A qualified pathologist/ cytotechnologist experienced in immunocytochemical procedures and trained on the interpretation of CINtec *PLUS* Cytology Kit stained slides must evaluate controls (if used) before interpreting results.

Interpretation of the test results may only be made by a certified professional in conjunction with the patient´s clinical history and additional diagnostic tests that have been performed.

For the interpretation of cervical cytology slides stained with the CINtec *PLUS* Cytology Kit, the slides should be evaluated with regard to the presence of cervical epithelial cells showing both cytoplasmic brown and nuclear red staining indicative of simultaneous $p16^{INK4a}$ and Ki-67 expression.

#### Positive Test Result

The presence of one or more cervical epithelial cells with co-localization of specific brown cytoplasmic immunostaining and specific red nuclear immunostaining within the same cell is regarded as a positive CINtec *PLUS* Cytology test result.

#### Negative Test Result

If no cervical epithelial cell shows simultaneous brown cytoplasmic immunostaining and red nuclear immunostaining, the CINtec *PLUS* Cytology test result is considered negative.

The presence of cervical epithelial cells that show immunoreactivity only for one but not both markers (such as brown staining for $p16^{INK4a}$ only or red staining for Ki-67 only) is not considered a positive test result for the CINtec *PLUS* Cytology Kit.

## LIMITATIONS

1. For professional use only. Special training is required for the performance of immunocytochemical procedures.
2. Evaluation of microscope slides stained with the CINtec PLUS Cytology Kit should be performed only by a certified professional who has been trained to interpret these test results.
3. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation and cytological criteria.
4. The interpretation of CINtec PLUS Cytology Kit staining results depends on the intensity and quality of the hematoxylin counterstaining. Deviation from the recommended reagents and incubation times requires validation by the customer as excessive or incomplete counterstaining may interfere with proper interpretation of results.
5. Conventional smear slides intended to be used for staining with the CINtec PLUS Cytology Kit shall be prepared by using SuperFrost® PLUS (Thermo Fisher Scientific) microscope glass slides and Safetex™ Cytology Fixative (Andwin Scientific), a cytological spray fixation reagent containing polyethylene glycol. Deviation from this recommendation requires validation by the customer.
6. The use of the ThinPrep® 3000 Processor is not recommended for preparation of ThinPrep® samples as the spray fixation procedure performed by the instrument may lead to substantial cell loss when slides prepared are stained with the CINtec PLUS Cytology Kit.
7. ThinPrep® Arcless Microscope Slides or ThinPrep® Microscope Slides FOR SPECIAL PROCESSING (Hologic Order # 70126-002) are required for the preparation of ThinPrep® samples for immunocytochemistry staining with the CINtec PLUS Cytology Kit on VENTANA BenchMark GX, XT and ULTRA automated slide stainers. ThinPrep® microscope slides with an imprinted screening area may lead to inconsistent staining results.
8. The manufacturer provides these antibodies/reagents at optimal dilution for use according to the instructions provided herein, for immunocytochemistry testing on prepared liquid-based cytology (LBC) slides or conventional smear slides. Any deviation from the recommended test procedures may invalidate declared expected results; appropriate controls should be employed and documented. Users who deviate from the recommended test procedures must accept responsibility for interpretation of patient results under these circumstances.

## TROUBLESHOOTING

1. If a reagent dispenser does not dispense fluid, check the priming chamber or meniscus for foreign materials or particulates, such as fibers or precipitates. If the dispenser is blocked, do not use the dispenser and contact your local support representative. Otherwise, re-prime the dispenser by aiming the dispenser over a waste container, removing the nozzle cap, and pressing down on the top of the dispenser.
2. If the positive control exhibits weaker staining than expected, check if the selected protocol matches the specific specimen type; for example, SurePath™ cytology preparations require longer cell conditioning time than ThinPrep® slides or conventional smear preparations. In addition, check to ensure that all dispenser barrels are clear from debris.
3. 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, check to ensure that all dispenser barrels are clear from debris.
4. If high background is observed, decrease incubation times in the staining protocols. In addition, check to ensure the Reaction Buffer bulk solution was formulated correctly.
5. If weak staining is observed, increase incubation times in the staining protocols. On a conventional smear slide, cell conditioning incubation time can also be adjusted.
6. The red precipitate used to indicate Ki-67 protein expression is alcohol soluble. If Ki-67 staining is weak or not present, ensure that alcohol-containing hematoxylin was not used and that the recommended post-processing procedure was followed according to the Post Processing Procedure - Mounting and Coverslipping instructions.
7. If sample washes off the slide, slides should be checked to ensure that the sample was prepared properly according to the Specimen Preparation section and recommended microscope slide type was used.
8. For corrective action, refer to the Staining Procedure section, the instrument Operator's Manual or contact your local support representative.

## PERFORMANCE CHARACTERISTICS

The performance of the CINtec PLUS Cytology Kit was evaluated though analytical specificity and reproducibility studies as described in the following section.

### Analytical Specificity

Analytical specificity of the CINtec PLUS Cytology Kit was evaluated by inhibition of the primary antibody clones E6H4 and 274-11 AC3 with the corresponding epitope-specific peptides. Liquid-based cytology (LBC) slides from a cell line overexpressing both antigens were used to assess the effect of specific peptide inhibition on the staining performance for both antibodies. Binding of the antibodies to their epitope-specific peptides led to inhibition of the antibodies which was detected by decreased staining compared to a no-peptide control. In addition, neither antibody was inhibited by the other antibody's epitope-specific peptide indicating that non-specific peptides will not inhibit these antibodies.

### Reproducibility

Reproducibility studies for the CINtec *PLUS* Cytology Kit were performed to demonstrate:

- Inter-lot reproducibility of the CINtec *PLUS* Cytology Kit;
- Intra-run and Inter-run reproducibility on the VENTANA BenchMark GX, XT and ULTRA automated slide stainers;
- Intra-platform reproducibility on the VENTANA BenchMark GX, XT and ULTRA automated slide stainers;
- Inter-platform reproducibility between the VENTANA BenchMark GX, XT and ULTRA automated slide stainers.

All studies met their pre-determined acceptance criteria.

## REFERENCES


1. Li J, Poi MJ, et al.. Regulatory mechanisms of tumor suppressor $P16^{INK4A}$ and their relevance to cancer. Biochemistry. 2011;50(25):5566-82.
2. Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. J Cell Physiol. 2000;182:311-22.
3. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda system. Terminology for reporting results of cervical cytology. JAMA. 2002;287:2114-9.
4. Birdsong G., Husain M., Faison T, et al. Cervicovaginal Cytology Based on the Papanicolaou Technique; Approved Guideline- Third Edition. CLSI document GP15-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.


NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

### Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for more information).

| | |
|---|---|
| GTIN | Global Trade Item Number |
| Rx only | For USA: Caution: Federal law restricts this device to sale by or on the order of a physician. |

## REVISION HISTORY

| Rev | Updates |
|---|---|
| E | Updates to Warnings and Precautions section. Updated to current template. |

## INTELLECTUAL PROPERTY



For USA: Rx only

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