digene® HC2 Sample Conversion Kit

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INTENDED USE

The *digene*® Hybrid Capture® 2 (HC2) Sample Conversion Kit is intended for use only in conjunction with cervical specimens collected in Hologic PreservCyt® Solution for processing and use with the *digene* HC2 HPV DNA Test and the *digene* HC2 High-Risk HPV DNA Test.

For Professional Use Only.

Read these instructions fully and carefully before using this kit. It is also important to read the instructions provided in the *digene* HC2 HPV DNA tests' instructions for use before proceeding.

These instructions are for manual testing only. For testing using the Rapid Capture® System, refer to the Rapid Capture System User Manual.

SUMMARY AND EXPLANATION

The *digene* HC2 Sample Conversion Kit consists of Sample Conversion Buffer, Specimen Transport Medium, Denaturation Reagent, and Indicator Dye. These reagents are used to pellet, resuspend, and denature cervical cells collected in PreservCyt Solution in order to test them using the *digene* HC2 HPV DNA tests. Refer to the *digene* HC2 HPV DNA tests' instructions for use for detailed instructions on assay procedure and performance for each test.

PRINCIPLE

Use of the *digene* HC2 Sample Conversion Kit with PreservCyt Solution allows both cytologic diagnosis (ThinPrep Pap Test) and the *digene* HC2 HPV DNA tests to be performed from the same specimen. After ThinPrep Pap Test slides are prepared according to instructions provided by Hologic, the remaining specimen volume is used to perform *digene* HC2 HPV DNA testing. There must be at least 4 ml of PreservCyt Solution remaining (from the original 20 ml) after the ThinPrep Pap Test slide is made. Otherwise, the specimen volume is inadequate for the *digene* HC2 HPV DNA tests, and therefore specimens should not be tested.

REAGENTS PROVIDED

1 x 100 ml
Sample Conversion Buffer: Buffered solution with Eosin Y and 0.05% (w/v) sodium azide.

1 x 30 ml
Specimen Transport Medium (STM): Contains 0.05% (w/v) sodium azide.

1 x 12 ml
Denaturation Reagent: Dilute sodium hydroxide (NaOH) solution.

1 x 0.35 ml
Indicator Dye: Contains 0.05% (w/v) sodium azide.

GLOSSARY OF SYMBOLS

	Consult instructions for use
REF	Catalog number
LOT	Batch code
	Manufacturer
IVD	In-vitro diagnostic medical device
	Use by
EC REP	Authorized representative in the European Community
RX ONLY	Caution: U.S. Federal law restricts this device to sale by or on the order of a licensed practitioner.
GTIN	Global Trade Item Number
Σ N	Contains sufficient amount for <N> samples

MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

digene HC2 HPV DNA Test[1]
digene HC2 High-Risk HPV DNA Test[1]

Swinging Bucket Centrifuge capable of 2,900 ± 150 x g and holding 10-ml or 15-ml conical tubes specified below

Repeating positive-displacement pipettor such as Eppendorf® Repeater® Pipette or equivalent

Disposable tips for Eppendorf Repeater Pipette or equivalent

5-ml serological pipettes or transfer pipettes

Vortex Mixer with cup attachment

65 ± 2°C water bath of sufficient size to hold either 1 Conversion Rack (36 x 21 x 9 cm) or specimen racks

Absorbent low-lint paper towels

Manual Vortex Procedure

Sarstedt brand 10-ml or VWR or Corning brand 15-ml conical-bottom polypropylene centrifuge tubes with caps

Multi-Specimen Tube Vortexer 2 Procedure

VWR or Corning brand 15-ml conical-bottom polypropylene centrifuge tubes with caps

Multi-Specimen Tube (MST) Vortexer 2[1]

Conversion Rack and Lid (specific for 15-ml conical tubes)[1]

Tube Sealer dispenser and cutting device[1]

DuraSeal® Tube Sealer Film (used with the MST Vortexer 2)[1]

[1]These items are available from QIAGEN.

All materials required but not supplied, as listed in the *digene* HC2 HPV DNA tests' instrutions for use, are required to test these specimens.

WARNINGS AND PRECAUTIONS

1. The following risk and safety phrases apply to components of the *digene* HC2 Sample Conversion Kit:

Denaturation Reagent:

Contains: sodium hydroxide. Danger! May be corrosive to metals. Causes severe skin burns and eye damage. Wear protective gloves/ protective clothing/ eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician.

Specimen Transport Medium

Warning! Causes mild skin irritation. Wear protective gloves/ protective clothing/ eye protection/ face protection.

Further information

Safety Data Sheets: www.qiagen.com/safety

2. PRESERVCYT SOLUTION: Contains methanol, which is poisonous. Consult the PreservCyt Solution product labeling for warnings and precautions.

3. Sodium azide is used as a preservative in some reagents. It has been reported that azides may react with lead and copper in plumbing to form explosive compounds. When disposing of decontaminated liquids, flush drains thoroughly with copious amounts of water to minimize buildup of metal azide compounds.

4. Observe all safety precautions listed in the *digene* HC2 HPV DNA tests' instructions for use when using the *digene* HC2 Sample Conversion Kit.

5. ALL SPECIMENS should be considered potentially infectious. No known testmethod can offer complete assurance that specimens will not transmit infection. It is recommended that human specimens be handled in accordance with the appropriate national/local biosafety practices. Use these biosafety practices with materials that contain or are suspected of containing infectious agents.

Storage and Handling Precautions

1. Wear powder-free gloves.

2. Store the *digene* HC2 Sample Conversion Kit at room temperature (15-30°C). Prior to initial use, the *digene* HC2 Sample Conversion Kit can be used until the expiration date indicated next to the symbol on the outer box label.

3. Store Denaturation Reagent at 2-8°C after addition of Indicator Dye. Once prepared, the Denaturation Reagent is stable for 3 months when stored at 2-8°C and should be labeled with the appropriate expiration date. If the color fades in this time frame, add 3 additional drops of Indicator Dye and shake by hand until contents are a uniform color.

PRESERVCYT SOLUTION SPECIMEN PREPARATION PROCEDURE

For testing using the Rapid Capture System, refer to the Rapid Capture System User Manual.

Notes:

- Processing a 4-ml aliquot of PreservCyt Solution produces enough material for 2 tests, when tested manually. The minimum volume that can be processed is 4 ml.
- Prepare PreservCyt Solution samples in batches of 36 or fewer; otherwise, pellets may become dislodged when decanting the supernatant. This is important for maintaining the integrity of the cell pellet during the decanting step. If preparing additional PreservCyt Solution vials, do not start to prepare them until after completing the preparation of the first batch.
- If using the MST Vortexer 2 or the RCS, *digene* HC2 Sample Conversion tubes or 15 ml VWR or Corning brand conical, polypropylene tubes with caps must be used for this procedure.

Reagent Preparation

To prepare the Denaturation Reagent (DNR), add 3 drops of Indicator Dye to the bottle of DNR and mix well. The solution should be a uniform, dark purple color. To determine volume requirements, use Table 1.

Table 1

No. of Tests	PreservCyt Volume	Conversion Buffer Volume
1-2	4 ml	0.4 ml
3	6 ml	0.6 ml
4	8 ml	0.8 ml
5	10 ml	1.0 ml
6	12 ml	1.2 ml

1. Label *digene* HC2 Sample Conversion Tube, a 10-ml conical Sarstedt brand tube, or a 15-ml VWR or Corning brand conical tube with the appropriate specimen identification number.

2. Handling one specimen at a time:

   a. Shake the PreservCyt vial vigorously by hand to resuspend cells and ensure homogeneity, or by vortexing each vial individually using a vortex mixer at maximum speed setting for approximately 5-10 seconds.

   b. Immediately, as cells settle very quickly, pipette the appropriate volume of the PreservCyt specimen into the labeled tube. Deliver the PreservCyt Solution to the bottom of the conical tube to minimize cellular material adhering to the inside of the tube.

3. Add the appropriate volume of Sample Conversion Buffer to each tube (see Table 1).

4. Recap and mix the contents of each tube thoroughly using a vortex mixer with cup attachment.

5. Centrifuge the tubes in a swinging bucket rotor at 2,900 ± 150 x g for 15 ± 2 minutes.

6. During centrifugation, prepare the Specimen Transport Medium (STM)/ Denaturation Reagent (DNR) mixture in a 2:1 ratio, according to Table 2.

   Note: Solution must be prepared fresh each day the test is being performed.

   a. To determine the total volume of STM/DNR mixture required, use the starting volume of the PreservCyt Solution specimen as a guide and then multiply the STM and DNR "per tube" volumes by the number of specimens to be processed.

Table 2

No. of Tests	PreservCyt Volume	STM Volume per tube for final STM + DNR Mixture*	DNR Volume per tube for final STM + DNR Mixture*	STM + DNR Mixture added per tube
1-2	4 ml	120 µl	60 µl	150 µl
3	6 ml	170 µl	85 µl	225 µl
4	8 ml	220 µl	110 µl	300 µl
5	10 ml	270 µl	135 µl	375 µl
6	12 ml	320 µl	160 µl	450 µl

* The volumes listed in these columns should not be added directly to the specimen tube.

   b. Mix the solution thoroughly by vortexing.

7. Remove tubes from the centrifuge one at a time and place into a rack or Conversion Rack. A pink/orange pellet should be present in the bottom of each tube.

   Note: Samples that do not have a visible pellet after centrifugation are not acceptable for testing and should be discarded.

8. Handling each tube individually:

   a. Remove the cap and set aside on a clean low-lint paper towel.

   b. Carefully decant the supernatant.

   c. Maintain the inverted tube position and gently blot (approximately 6 times) on absorbent low-lint paper towels to remove the excess liquid. Use a clean area of the towel each time. **Do not** allow the cell pellet to slide down the tube during blotting.

   d. Place the tube in a rack or the Conversion Rack.

Notes:

- Do not blot in the same area of the absorbent low-lint paper towel.
- It is important to remove the maximum amount of PreservCyt Solution by blotting. However, it is normal to see residual PreservCyt Solution after blotting.

9. Repeat the steps for all of the samples until all are decanted.

VORTEXING

Manual Vortexing Procedure

1. Add the specified volume of the STM/DNR mixture to each tube according to Table 2. Recap each tube and resuspend the pellets individually by vortexing each tube for at least 30 seconds at the highest speed setting. If a pellet is difficult to resuspend, vortex for an additional 10-30 seconds or until the pellet floats loose from the bottom of the tube. If a pellet remains undissolved after additional vortexing (a total of 2 minutes maximum), note the sample identification and proceed to the next step.

2. Place tubes in 65 ± 2°C water bath for 15 ± 2 minutes. Ensure that the water level is sufficient to cover all liquid in the tubes.

3. Remove the rack with specimens from the water bath and vortex samples individually for 15-30 seconds.

   Note: Make sure that all pellets are completely resuspended at this point. Discard samples that still have visible pellets as they are not acceptable for testing.

4. Return the rack to the 65 ± 2°C water bath and continue denaturation for another 30 ± 3 minutes.

5. Proceed to the *Hybridization Step* as described in the *digene* HC2 HPV DNA tests' instructions for use or see *Optional Stop Point* for storage and treatment of denatured samples.

Multi-Specimen Tube Vortexer 2 Procedure

Notes:

- The Multi-Specimen Tube (MST) Vortexer 2 method is validated for the processing of PreservCyt Solution specimens following centrifugation and decanting of the supernatant.
- The MST Vortexer 2 procedure has not been validated for vortexing PreservCyt Solution specimens with Sample Conversion Buffer prior to centrifugation.
- Only the MST Vortexer 2 is designed for PreservCyt Solution specimen processing. The Multi-Specimen Tube (MST) Vortexer I is not designed for PreservCyt Solution processing because it is incompatible with the Conversion Rack and Lid. Therefore, do not use the Conversion Rack with the MST Vortexer I.
- The Conversion Rack and Lid are specifically designed to accommodate VWR or Corning brand 15-ml conical tubes. The user should use only one tube type on the Conversion Rack at a time. Other brands are not validated for use.
- Strict adherence to the specified vortexing times of the Conversion Rack and Lid is required. This procedure standardizes the mixing speed, times, and process, eliminating the need to visually check for cell pellets required when performing resuspension with a vortexer.
- The Conversion Rack and Lid cannot be used to vortex the *digene* HC2 HPV DNA tests' kit Controls, Calibrators, or Quality Controls. The height of the STM tubes prevents adequate vortexing using the Conversion Rack and Lid.

1. After blotting each labeled 15-ml conical tube, place each in its proper position in the Conversion Rack.

2. Add the specified volume of the STM/DNR mixture to each tube according to Table 2.

3. Cover the 15-ml conical tubes with DuraSeal Tube Sealer Film by pulling the film over the tubes in the rack.

4. Place the rack lid over the film-covered tubes and lock the lid into place with the two side clamps. Cut the film with the cutting device after the lid is securely fastened.

5. Move the red-handled lever up so that it is in a horizontal position.

6. Place the Conversion Rack and Lid on the MST Vortexer 2 so that the largest notched corner of the Conversion Rack is located in the right front corner. Position the rack and lid on the MST Vortexer 2 platform so that it fits securely within the guides. Secure the rack in place by pushing the red-handled lever down to the vertical position. This will lock the rack in place.

7. Verify that the speed setting is at 100 (maximum speed) and the Pulser button is in the OFF position.

8. Turn the Vortexer power switch to the ON position. **Vortex the tubes for 30 seconds**.

9. Turn the Vortexer power switch to the OFF position.

10. Remove the Conversion Rack and Lid from the MST Vortexer 2 by lifting up on the red-handled lever.

11. Place the rack in the 65 ± 2°C water bath for 15 ± 2 minutes. Be sure the water level completely covers all liquid in all of the tubes.

12. After the 15-minute incubation, remove the rack with specimens from the water bath.

13. To prevent splashing, dry the rack of excess water prior to placing it on the MST Vortexer 2.

14. Secure the Conversion Rack and Lid on the MST Vortexer 2 as described in *Step 6*.

15. Verify speed setting is at 100, and turn the vortexer power switch to the ON position. **Vortex the tubes for 1 minute**.

16. Turn the power switch to the OFF position.

17. Return the rack to the 65 ± 2°C water bath, and continue denaturation for 30 ± 3 minutes.

18. Remove rack from water bath, dry the rack, and secure on the vortexer. Make sure that the speed setting is at the maximum speed of 100.

19. Turn the Vortexer power switch to the ON position. **Vortex for 10 seconds at the maximum setting**.

20. Turn the Vortexer power switch to the OFF position. Remove the rack.

21. Immediately place the Conversion Rack on the bench top and release the latches. Lift the rack lid approximately 1 cm and move gently left and right to release any tubes that may have adhered to the DuraSeal tube sealer film. Remove the rack lid by lifting it straight up until it clears the Conversion Rack. Carefully peel the DuraSeal tube sealer film from the rack lid and discard.

22. Proceed to the *Hybridization Step* as described in the *digene* HC2 HPV DNA tests' instructions for use or see *Optional Stop Point* for storage and treatment of denatured samples.

Optional Stop Point

After denaturation, converted PreservCyt specimens may be stored at 2 - 8°C overnight or at -20°C for up to 3 months. For overnight refrigeration, specimens may be left in the Conversion Rack with new DuraSeal film and Rack Lid replaced. Prior to storage at -20°C, the Rack Lid and DuraSeal film must be removed, and caps must be placed on the tubes. If the manual vortex procedure was used, place the rack of capped tubes in the desired storage temperature. In either case, the specimens must be equilibrated to room temperature (20 - 25°C) and thoroughly vortexed before proceeding to the Hybridization step.

Note: Do not store or ship denatured samples on dry ice.

A maximum of 3 freeze/thaw cycles may be performed with a maximum of 2 hours at room temperature during each thaw cycle. Refer to the *digene* HC2 HPV DNA tests' instructions for use for detailed instructions on assay performance.

LIMITATIONS OF THE PROCEDURE

1. IVD

2. PreservCyt Solution specimens containing less than 4 ml are considered inadequate for *digene* HC2 HPV DNA tests'.

3. Prepare PreservCyt Solution specimens in batches of 36 or fewer. If processing more than 36 specimens at the same time, the additional pellets formed after centrifugation may loosen and be inadvertently discarded during the decanting step.

4. Refer to the *digene* HC2 HPV DNA tests' instructions for use for other limitations.

ORDERING INFORMATION

Use the QIAGEN Contact Information Sheet provided with this product to contact your local QIAGEN representative.

digene HC2 Sample Conversion Kit

digene HC2 High-Risk HPV DNA Test

digene HC2 Sample Conversion Tubes

Multi-Specimen Tube (MST) Vortexer 2 (120V or 240V)

Conversion Rack and Lid

Tube Sealer Dispenser and cutting device

DuraSeal Tube Sealer Film

TRADEMARKS

Limited License Agreement for *digene* HC2 Sample Conversion Kit Instructions For Use
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SIGNIFICANT CHANGES:

- Updated Formatting of IFU.