digene® HC2 Sample Conversion Kit Instructions For Use

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The *digene* HC2 Sample Conversion Kit is intended for use only in conjunction with cervical specimens collected in PreservCyt® Solution

For use with:

- *digene* HC2 High-Risk HPV DNA Test
- *digene* HC2 HPV DNA Test
- *digene* HC2 CT-ID DNA Test
- *digene* HC2 GC-ID DNA Test

CE

REF 5127-1220

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QIAGEN®

Sample & Assay Technologies

Key changes from previous instructions for use revision:

- Updated product branding and layout

Contents

Intended Use

For in vitro diagnostic use.

For professional use only.

The *digene* HC2 Sample Conversion Kit is intended for use only in conjunction with cervical specimens collected in PreservCyt Solution for processing and use with the *digene* HC2 HPV DNA Test, the *digene* HC2 High-Risk HPV DNA Test, the *digene* HC2 CT-ID DNA Test, and the *digene* HC2 GC-ID DNA Test.

Summary and Explanation

The *digene* HC2 Sample Conversion Kit consists of Sample Conversion Buffer, Specimen Transport Medium (STM), 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 HC2 DNA tests. Refer to the instructions for use of the respective HC2 DNA test for additional instructions.

Principle of the Procedure

Use of the *digene* HC2 Sample Conversion Kit with PreservCyt specimens allows the performance of both the cytological diagnosis with the ThinPrep® Pap Test and the HC2 DNA tests using the same specimen.

After the ThinPrep Pap Test slides are prepared according to the instructions for use provided by the manufacturer, the remaining specimen volume is used to perform HC2 DNA testing. At least 4 ml of PreservCyt Solution must remain after the ThinPrep Pap Test slide has been prepared. Otherwise, the specimen volume is inadequate for testing with the HC2 DNA tests and a false-negative result could occur.

Processing a 4 ml aliquot of a PreservCyt specimen produces enough material for 2 tests when performing manual testing or enough material for 1 test when performing Rapid Capture® System (RCS)-automated testing. Each additional 2 ml of PreservCyt specimen processed is sufficient volume for 1 additional test, regardless of testing method used.

Materials Provided

Kit contents

***digene* HC2 Sample Conversion Kit**		**(200)***
Catalog no.		**5127-1220**
Indicator Dye Contains 0.05% (w/v) sodium azide	INDIC	0.35 ml
Denaturation Reagent† Dilute sodium hydroxide (NaOH) solution	REAG DENAT	12 ml
Sample Conversion Buffer Buffered solution with Eosin Y and 0.05% (w/v) sodium azide	BUF SAMP CONV	100 ml
Specimen Transport Medium Contains 0.05% (w/v) sodium azide	MED SPEC TRANS	30 ml

* The number of samples that can be processed using the *digene* HC2 Sample Conversion Kit is based on the starting volume of PreservCyt specimen. If more than 4 ml of PreservCyt specimen is used for sample processing, the number of samples processed will be reduced.

† See "Warnings and Precautions," page 7, for health and safety information.

Materials Required but Not Provided

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Swinging bucket centrifuge capable of 2900 ± 150 x *g* and holding 10 ml or 15 ml conical, polypropylene centrifuge tubes
- 65 ± 2°C waterbath of sufficient size to hold a specimen rack [21 cm wide x 32 cm deep x 18 cm high (8.25 x 12.25 x 4.9 in.)]
- Vortexer with cup attachment

- Repeating positive displacement pipet, such as Eppendorf® Repeater® pipet*
- Disposable tips for repeating positive displacement pipet (5 and 2.5 or 1.25 ml)
- 5 ml disposable serological pipets or transfer pipets
- Kimtowels® wipers or equivalent low-lint paper towels*

Materials required if performing the resuspension and denaturation step using a vortexer

- *digene* HC2 Sample Conversion Tubes†, 10 ml Sarstedt® conical tubes with caps, or 15 ml VWR® or Corning® conical, polypropylene tubes with caps*
- Tube rack to hold 10 ml or 15 ml conical tubes

Materials required if performing the resuspension and denaturation step using the Multi-Specimen Tube (MST) Vortexer 2

- *digene* HC2 Sample Conversion Tubes† or 15 ml VWR or Corning brand conical, polypropylene tubes with caps‡
- MST Vortexer 2†
- Conversion Rack and Lid†
- Tube Sealer Dispenser and cutting device†
- DuraSeal™ tube sealer film*†

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

† Only equipment and materials validated with the *digene* HC2 Sample Conversion Kit are available from QIAGEN.

‡ Only the specified tubes can be used with the MST Vortexer 2 or the RCS.

Warnings and Precautions

For in vitro diagnostic use.

Read all instructions carefully before using the test.

Warnings

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Specimens

WARNING 	Specimens may contain infectious agents and should be handled accordingly. Consider all specimens potentially infectious.

No known test method can offer complete assurance that specimens will not transmit infection. It is recommended that human specimens be handled in accordance with the appropriate national and local biosafety practices. Use these biosafety practices with materials that contain or are suspected of containing infectious agents.

These precautions include, but are not limited to, the following:

- Do not pipet by mouth.
- Do not smoke, eat, or drink in areas where reagents or specimens are handled.
- Wear disposable powder-free gloves while handling reagents or specimens. Wash hands thoroughly after performing the test.
- Clean and disinfect all spills of specimens using a tuberculocidal disinfectant such as 0.5% v/v sodium hypochlorite, or other suitable disinfectant (1, 2).

- Decontaminate and dispose of all specimens, reagents, and other potentially contaminated materials in accordance with national and local regulations.

Following denaturation and incubation, the specimens are no longer considered infectious (3); however, lab personnel should still adhere to national and local precautions.

PreservCyt Solution contains methanol which is poisonous. Consult the PreservCyt Solution product labeling for warnings and precautions.

Sodium azide

Some reagents contain sodium azide. Sodium azide has been reported to form lead or copper azide in laboratory plumbing. These azides may explode upon percussion, such as hammering. To prevent the formation of lead or copper azide, flush drains thoroughly with water after disposing of solutions containing sodium azide. To remove contamination from old drains suspected of azide accumulation, the U.S. Occupational Safety and Health Administration recommends the following:

1. Siphon liquid from trap using a rubber or plastic hose.
2. Fill with 10% v/v sodium hydroxide solution.
3. Allow to stand for 16 hours.
4. Flush well with water.

Safety and risk statements for components

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

Denaturation Reagent

Contains: sodium hydroxide. Danger! Causes severe skin burns and eye damage. May be corrosive to metals. Dispose of contents/ container to an approved waste disposal plant. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF ON SKIN (or hair): Remove/ Take off immediately all contaminated clothing. Rinse skin with water/ shower. Immediately call a

POISON CENTER or doctor/ physician. Store locked up. Wear protective gloves/ protective clothing/ eye protection/ face protection.

Specimen Transport Medium

Warning! Causes mild skin irritation. If skin irritation occurs: Get medical advice/ attention.

Further information

Safety Data Sheets: www.qiagen.com/safety

Precautions

The user must always adhere to the following precautions when using the *digene* HC2 Sample Conversion Kit:

- Do not use the reagents beyond the expiration date indicated next to the ⌛ symbol on the outer box label or the expiration date of the prepared reagents.
- Wear powder-free gloves.

Reagent Storage and Handling

Kit components

Store the *digene* HC2 Sample Conversion Kit at 15–30°C. The Denaturation Reagent and Indicator Dye may be stored at 2–30°C, as desired. All reagents provided are ready-to-use, except the Denaturation Reagent (DNR) and the STM/DNR mixture.

Prepared reagents

Once prepared, the DNR is stable for 3 months when stored at 2–8°C.

Once prepared, the DNR/STM mixture must be used the day it was prepared.

Specimen Collection and Preparation

Collect specimens in the routine manner, and prepare the ThinPrep Pap Test slides according to the instructions for use provided by the manufacturer. At least 4 ml of PreservCyt specimen must remain for the *digene* HC2 Sample Conversion Kit. Specimens with less than 4 ml after the Pap Test has been prepared contain insufficient material and could cause a false-negative result in the HC2 DNA test.

Following collection, store PreservCyt specimens for up to 3 months at 2–30°C prior to sample preparation for the HC2 DNA tests. PreservCyt specimens cannot be frozen.

The result of manual sample preparation using the *digene* HC2 Sample Conversion Kit is a denatured sample ready to proceed to the hybridization step of the HC2 DNA test.

Procedure

Important points before starting:

- The minimum volume of PreservCyt specimen required is 4 ml.
- No more than 36 PreservCyt specimens may be processed at one time. The additional time necessary to process more than 36 specimens at one time increases the likelihood of the cell pellet becoming dislodged during the decanting of the supernatant prior to resuspension. The cell pellet becoming dislodged may produce an inaccurate test result.
- 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

Denaturation Reagent

1. **Add 3 drops of Indicator Dye to the bottle of Denaturation Reagent.**
2. **Mix thoroughly. The DNR should be a uniform, dark purple color.**
3. **Label the DNR with the new expiration date.**

   Notes:

   - Once prepared, the DNR is stable for 3 months when stored at 2–8°C.
   - If the color fades, add 3 additional drops of Indicator Dye and mix thoroughly before using.

STM/DNR mixture

Important points before starting:

- Prepare during the centrifugation step.
- The STM/DNR mixture is prepared in a 2:1 ratio.

1. **Determine the total volumes of STM and DNR required. Using the PreservCyt specimen volume to be prepared, multiply the STM and DNR volumes by the number of specimens to be prepared according to Table 1, below.**

Table 1. Preparation of STM/DNR

Number of tests to be performed per specimen	PreservCyt specimen volume to be prepared	STM volume per specimen	DNR volume per specimen
1–2	4 ml	120 µl	60 µl
3	6 ml	170 µl	85 µl
4	8 ml	220 µl	110 µl
5	10 ml	270 µl	135 µl
6	12 ml	320 µl	160 µl

2. **Label a new, disposable container as "STM/DNR mixture".**
3. **Add the determined volumes of each reagent to the container and cap the container.**
4. **Mix the solution thoroughly by vortexing.**
5. **Label the DNR/STM mixture with the expiration date.**

   Note: Once prepared, the DNR/STM mixture must be used the day it was prepared.

Centrifugation

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

   Important: No more than 36 PreservCyt specimens may be processed at one time.

2. **Handling one specimen at a time, shake each PreservCyt specimen vial vigorously by hand or vortex each vial individually using a vortexer at maximum speed setting until the cells appear to be homogenously dispersed.**

3. **Immediately, as cells settle very quickly, pipet the applicable volume of the PreservCyt specimen into the labeled tube.**

   Deliver the PreservCyt specimen to the bottom of the conical tube to minimize the cellular material adhering to the inside of the tube.

4. **Determine the correct volume of Sample Conversion Buffer to add to each tube according to Table 2, below, and add the specified amount of Sample Conversion Buffer to each sample.**

Table 2. Sample Conversion Buffer addition

Number of tests	PreservCyt specimen volume to be prepared	Sample Conversion Buffer volume to be added
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

5. **Cap and thoroughly mix each tube using a vortexer with cup attachment.**

   Important: Do not use the MST Vortexer 2 to mix the tubes. The MST Vortexer 2 has not been validated for vortexing PreservCyt samples with Sample Conversion Buffer prior to centrifugation.

6. **Centrifuge the tubes in a swinging bucket rotor at 2900 ± 150 x *g* for 15 ± 2 minutes.**

   During centrifugation, prepare the STM/DNR mixture (see "Reagent preparation," page 11).

7. **One at a time, remove a tube from the centrifuge, inspect the tube for a visible pellet, and place the tube into a rack or a Conversion Rack.**

   Important: A pink/orange pellet should be present in the bottom of each tube. Discard samples that do not have a visible pellet after centrifugation as they are not acceptable for testing.

8. **Proceed to "Decanting," starting on page 14.**

Decanting

1. **Handling only one sample, remove the cap and set the cap on a clean Kimtowels wiper or equivalent low-lint paper towel.**
2. **Carefully decant the supernatant.**
3. **Using a clean area of a Kimtowels wipers or equivalent low-lint paper towels each time and maintaining the inverted tube position, gently blot approximately 6 times until liquid no longer drips from the tube. Do not allow the cell pellet to slide down the tube during blotting.**

   Important: Do not blot in the same area of the paper towel more than once.

   Important: Remove the maximum amount of sample liquid by blotting; however, it is normal to see residual sample liquid after blotting.
4. **Place the tube in a rack or a Conversion Rack.**
5. **Repeat the steps for all of the samples until all are decanted.**
6. **Proceed to "Resuspension and denaturation," starting on page 15.**

Resuspension and denaturation

Use either the "Resuspension and denaturation using a vortexer" procedure, page 15, or the "Resuspension and denaturation using a MST Vortexer 2" procedure, page 16, to resuspend and denature the sample.

Resuspension and denaturation using a vortexer

1. **Add the specified volume of the STM/DNR mixture to each tube according to Table 3, below.**

Table 3. STM/DNR addition

Number of tests	PreservCyt specimen volume to be prepared	STM/DNR mixture to be added
1–2	4 ml	150 µl
3	6 ml	225 µl
4	8 ml	300 µl
5	10 ml	375 µl
6	12 ml	450 µl

2. **Cap each tube with the cap that was set aside during the decanting step, 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 is not dissolved after up to 2 minutes of additional vortexing, note the sample identification and proceed to the next step.

3. **Place the tubes in a rack and place the rack in a 65 ± 2°C waterbath for 15 ± 2 minutes.**

   Make sure that the water level is sufficient to cover all the liquid in the tubes.

4. **Remove the rack from the waterbath, and vortex the samples individually for 15–30 seconds.**

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

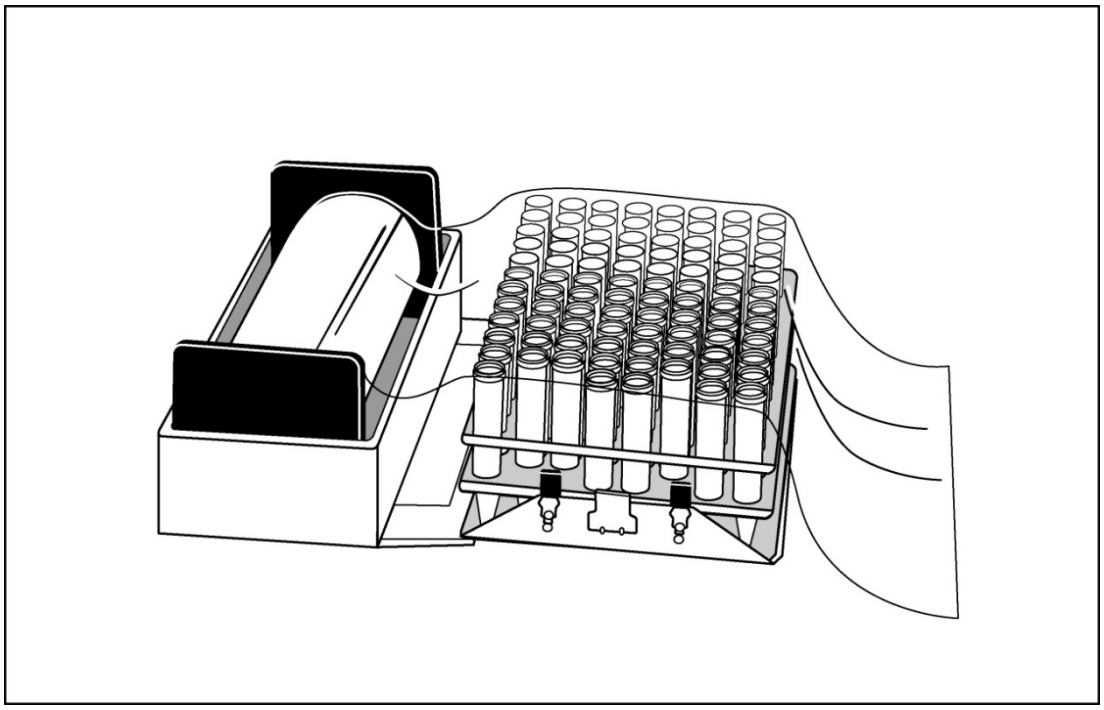
5. **Return the rack to the 65 ± 2°C waterbath, and continue the denaturation for another 30 ± 3 minutes.**
6. **Once the incubation is complete, remove the rack from the waterbath.**
7. **Proceed to the hybridization step of the respective HC2 DNA test or store the samples according to the "Optional Stop Point," page 20.**

Resuspension and denaturation using a MST Vortexer 2

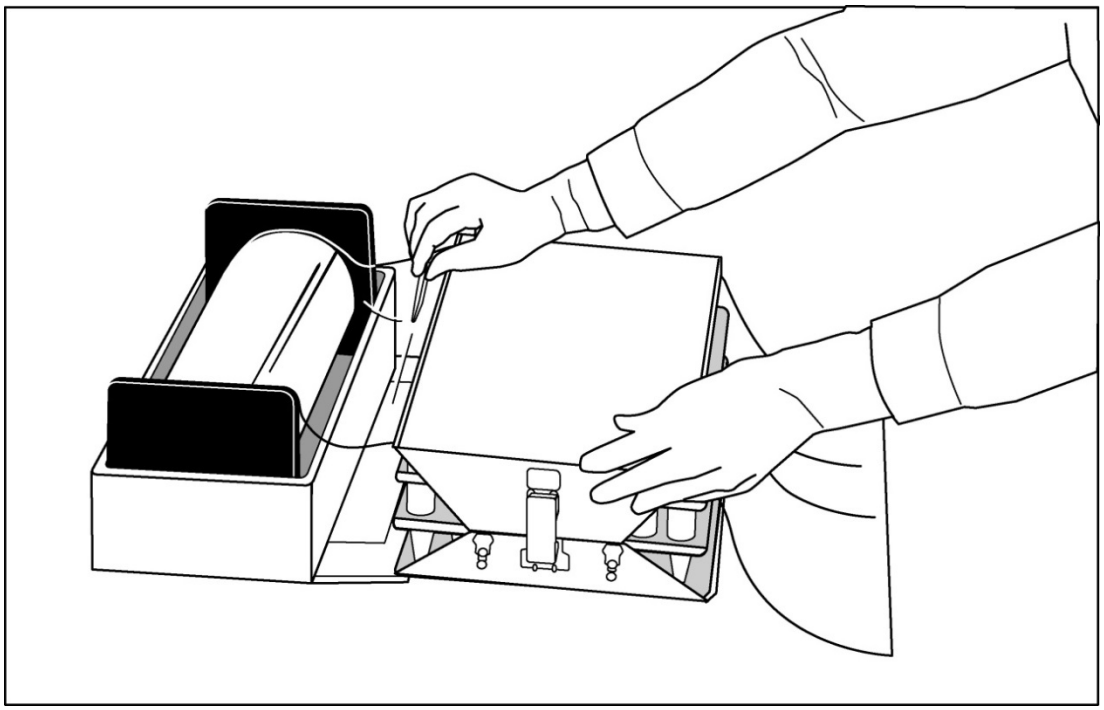
Important points before starting:

- The MST Vortexer 2 procedure is validated for the processing of PreservCyt samples following centrifugation and decanting of the supernatant.
- The MST Vortexer 2 is compatible with the Conversion Rack, which is designed for PreservCyt sample processing. The Conversion Rack is specifically designed to accommodate the *digene* HC2 Sample Conversion Tubes and 15 ml VWR or Corning brand conical, polypropylene tubes with caps. Other tubes are not validated for use with the Conversion Rack.
- Strict adherence to the specified vortexing times 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 cannot be used when vortexing the HC2 DNA test calibrators or quality controls. The height of the tubes prevents adequate vortexing using the Conversion Rack.
- To secure the Conversion Rack on the MST Vortexer 2, place the Conversion Rack on the MST Vortexer 2 so that the largest diagonal corner of the Conversion Rack is located in the right front corner. Position the Conversion Rack on the platform so that it fits securely within the guides. Secure the Conversion Rack in place by pushing the red lever down to the vertical position; this will lock the Conversion Rack in place.

1. **Add the specified volume of the STM/DNR mixture to each tube according to Table 3, above.**
2. **Cover the tubes with DuraSeal tube sealer film by pulling the film over the tubes in the Conversion Rack.**

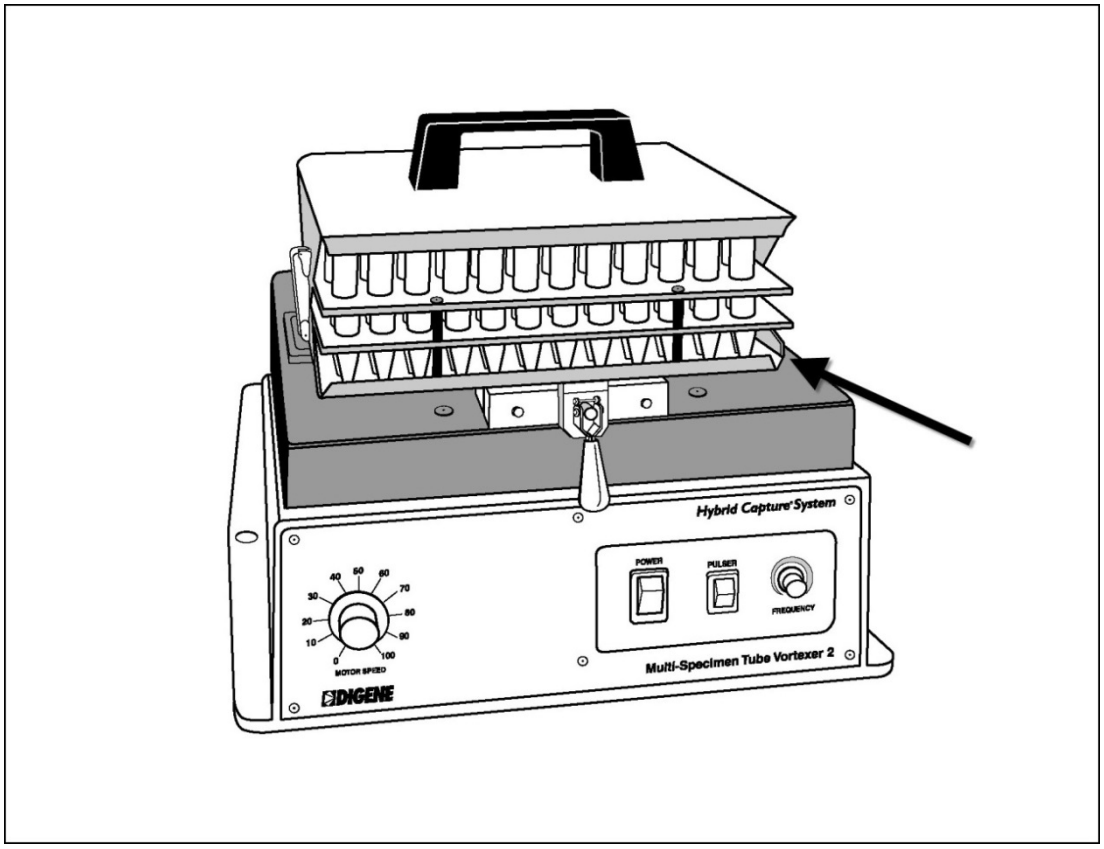


3. **Place the rack lid over the film-covered tubes and lock into place with the 2 side clamps. Cut the film with a cutting device.**



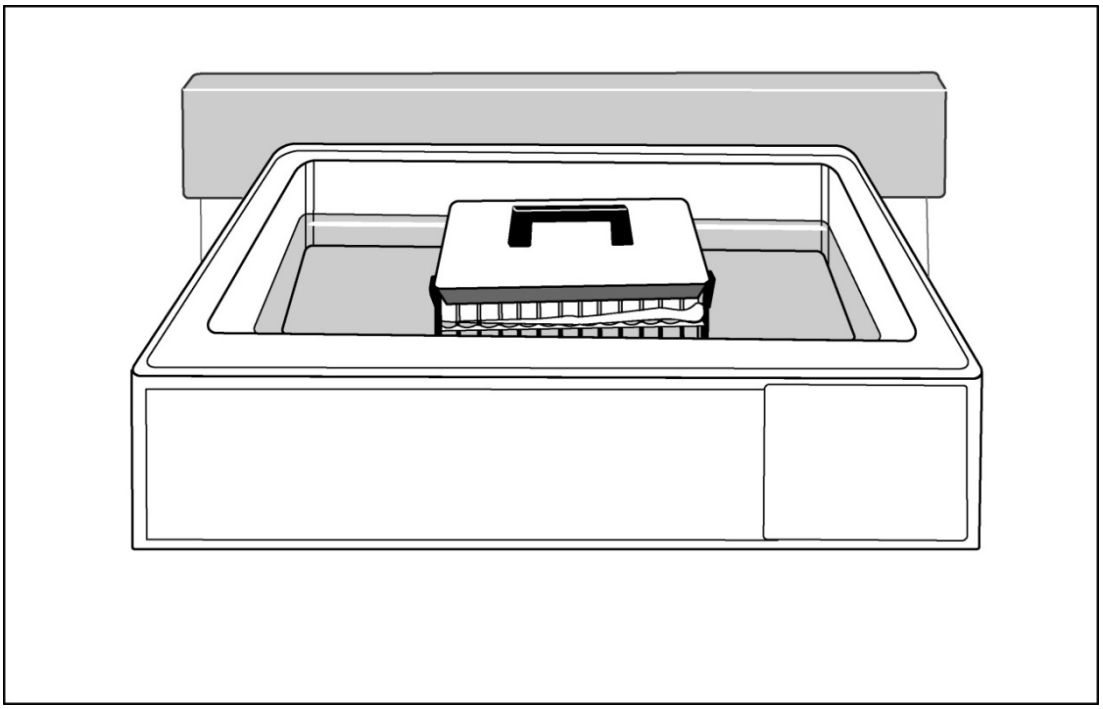
4. **Move the red lever up so that it is in a horizontal position.**

5. **Secure the Conversion Rack on the MST Vortexer 2.**

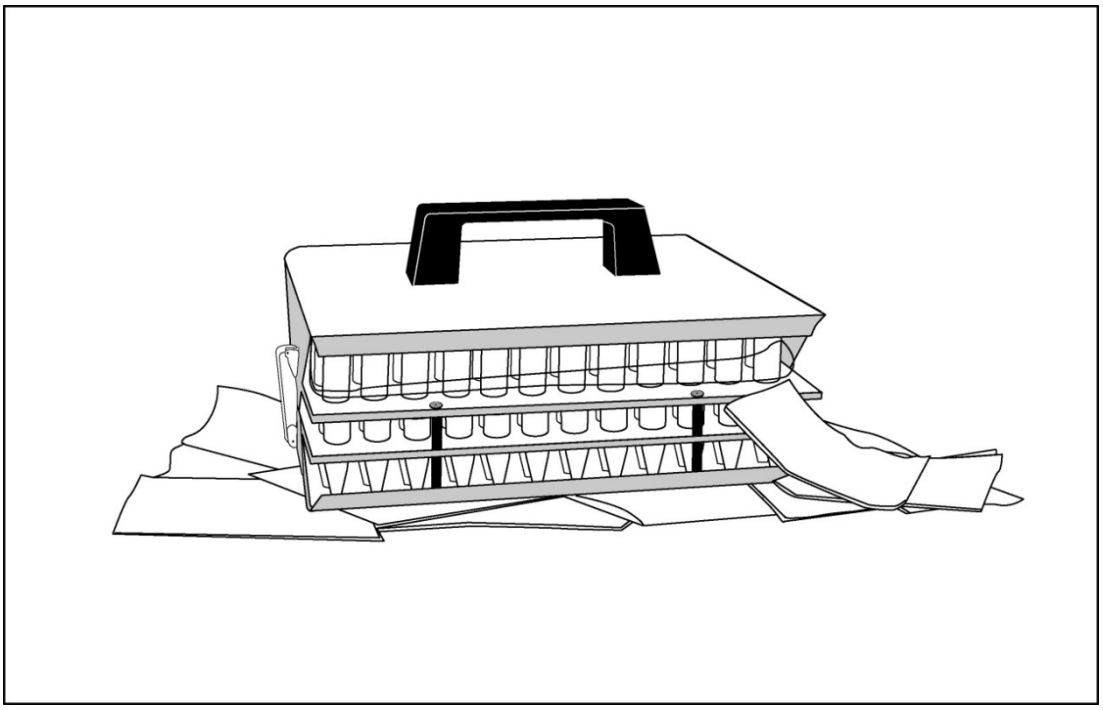


6. **Make sure that the MST Vortexer 2 speed setting is at 100 (maximum speed) and the Pulser toggle switch is in the OFF position.**
7. **Power ON the MST Vortexer 2 and vortex the tubes for 30 seconds. Power OFF the MST Vortexer 2.**
8. **Remove the Conversion Rack from the MST Vortexer 2 by lifting the red lever up to the horizontal position.**

9. **Place the Conversion Rack in the 65 ± 2°C waterbath for 15 ± 2 minutes.**
   Make sure that the water level completely covers all liquid in all of the tubes.



10. **Remove the Conversion Rack from the waterbath and, to prevent splashing, dry the Conversion Rack of excess water prior to placing it on the MST Vortexer 2.**



11. **Secure the Conversion Rack on the MST Vortexer 2.**

    Make sure that the speed setting is at the maximum speed of 100.

12. **Power ON the MST Vortexer 2, and vortex the tubes for 1 minute. Power OFF the MST Vortexer 2.**
13. **Return the Conversion Rack to the 65 ± 2°C waterbath, and continue the denaturation for 30 ± 3 minutes.**
14. **Remove the Conversion Rack from the waterbath, dry the Conversion Rack, and secure it on the MST Vortexer 2.**

    Make sure that the speed setting is at the maximum speed of 100.

15. **Power ON the MST Vortexer 2, and vortex the tubes for 10 seconds. Power OFF the MST Vortexer 2 and remove the Conversion Rack.**
16. **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.**
17. **Proceed to the hybridization step of the respective HC2 DNA test or store the samples according to the "Optional Stop Point," page 20.**

Optional Stop Point

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

All prepared samples may be stored at 2–8°C overnight or at –20°C for up to 3 months. A maximum of 3 freeze/thaw cycles may be performed with a maximum of 2 hours at room temperature during each thaw cycle.

For overnight storage at 2–8°C in the specimen rack, cover the samples with DuraSeal tube sealer film and replace the rack lid.

For storage at –20°C in the specimen rack, remove the rack lid and the DuraSeal tube sealer film, and place an appropriate cap on the tubes.

Limitations

- PreservCyt specimens containing less than 4 ml are inadequate for sample preparation with the *digene* HC2 Sample Conversion Kit and for testing with the HC2 DNA tests.
- Refer to the instructions for use of the respective HC2 DNA test for additional instructions and limitations.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen. com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Cited references

1. Centers for Disease Control (1987) Recommendations for prevention of HIV transmission in health-care settings. MMWR Morb. Mortal. Wkly. Rep. **36(Suppl 2)**, 3S.
2. Sehulster, L.M., Hollinger, F.B., Dreesman, G.R., and Melnick, J.L. (1981) Immunological and biophysical alteration of hepatitis B virus antigens by sodium hypochlorite disinfection. Appl. Environ. Microbiol. **42(5)**, 762.
3. Martin, L.S., McDougal, J.S., and Loskoski, S.L. (1985) Disinfection and inactivation of the human T lymphotropic virus type III/lymphadenopathy-associated virus. J. Infect. Dis. **152(2)**, 400.

Symbols

The following symbols are used in these instructions for use:

Symbol	Symbol definition
	Contains sufficient for 200 samples
	In vitro diagnostic medical device
	Catalog number
	Manufacturer
	Authorized representative in the European Community
	Use by
	Consult instructions for use

Contact Information

Use the QIAGEN contact information sheet provided with this product to contact your local QIAGEN representative.

Ordering Information

Product	Contents	Cat. no.
digene HC2 Sample Conversion Kit	Kit for sample preparation of PreservCyt specimens for use with the HC2 DNA tests	5127-1220
digene HC2 Sample Conversion Tubes	15 ml conical tubes (500 tubes)	6000-5026
Multi-Specimen Tube (MST) Vortexer 2	Multi-Specimen tube vortex, 120 V	6000-5021
Multi-Specimen Tube (MST) Vortexer 2	Multi-Specimen tube vortex, 240 V	6000-5022
Conversion Rack and Lid	Specimen rack for use with the MST Vortexer 2 and RCS	6000-5017
Tube Sealer Dispenser	Dispenser of tube sealant	6000-5004
DuraSeal Tube Sealer Film	Roll of tube sealant	6000-5003
digene HC2 High-Risk HPV DNA Test	Test kit for detection of 13 high-risk HPV types (96 tests)	5197-1330
digene HC2 HPV DNA Test	Test kit for detection of 13 high-risk and 5 low-risk HPV types (96 tests)	5196-1330
digene HC2 CT-ID DNA Test	Test kit for detection of *Chlamydia trachomatis* (CT) DNA from cervical specimens (96 tests)	5135-1330
digene HC2 GC-ID DNA Test	Test kit for detection of *Neisseria gonorrhoeae* (GC) DNA from cervical specimens (96 tests)	5140-1330

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This product and its method of use are covered by one or more of the following patents:

Hybrid Capture technology is covered by European Patent No. 0 667 918 registered in Austria, Belgium, Switzerland, Liechtenstein, Germany, Denmark, Spain, France, United Kingdom, Greece, Ireland, Italy, Luxembourg, Netherlands, and Sweden.

U.S. Hybrid Capture Patent

6,228,578B1

U.S. HPV Patents

5,643,715 • 5,712,092 • 5,876,922 • 5,952,487 • 5,958,674 • 5,981,173

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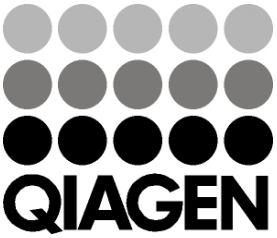

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