

Urine - Based XMRV RT-PCR Detection Kit
Product # 34700

Product Insert

Xenotropic murine leukemia virus-related virus (XMRV) belongs to the family Retroviridae and the genus gammaretrovirus. The virus was first described in 2006 and has since been isolated from human biological samples. XMRV has a single-stranded RNA genome that replicates through a DNA intermediate. The virus gets its name due to its close relationship with the murine leukemia viruses (MuLVs). The viral genome is approximately 8100 nucleotides in length and is 95% identical with several endogenous retroviruses of mice. While gammaretroviruses have well-characterized oncogenic effects in animals, they have not been shown to cause human cancers. However, XMRV was recently discovered in human prostate cancers and is the first gammaretrovirus known to infect humans. In addition to prostate cancer, a possible association with chronic fatigue syndrome has been reported, however it has yet to be established whether XMRV is a cause of this disease.

The causal role of XMRV in cancer has yet to be established and the virus does not appear to be capable of transforming cells directly. In prostate cancer, XMRV protein has been found in tumour-associated but nonmalignant stromal cells, but not in the actual prostate cancer cells. This raises the possibility that the virus may support tumorigenesis. In other studies, XMRV proteins and nucleic acids were found in malignant cells.

Principle of the Test

Norgen's Urine-Based XMRV RT-PCR Detection Kit constitutes a ready-to-use system for the isolation and detection of XMRV viral RNA using end-point RT-PCR. The kit first allows for the isolation of total RNA, including viral RNA, from the urine samples using spin-column chromatography based on Norgen's proprietary resin. The viral RNA is isolated free from inhibitors, and can then be used as the template in a RT-PCR reaction for XMRV detection using the provided XMRV Master Mix. The XMRV Master Mix contains reagents and enzymes for the specific amplification of a 300 bp region of XMRV. In addition, Norgen's Urine-Based XMRV RT-PCR Detection Kit contains a second heterologous amplification system to identify possible PCR inhibition and/or inadequate isolation. The amplification and detection of either the XMRV *Isolation Control (IsoC)* or the *PCR control (PCRC)* does not reduce the detection limit of the analytical XMRV PCR. The kit is designed to allow for the testing of 24 samples.

Kit Components:

| Component | Contents |
|--|----------------|
| RNA Lysis Solution | 3 x 90 mL |
| RNA Wash Solution | 24 mL |
| RNA Elution Solution | 6 mL |
| Mini Filter Spin Columns | 24 |
| Collection Tubes | 24 |
| Elution tubes (1.7 mL) | 24 |
| XMRV 2x RT-PCR Master Mix | 0.35 mL |
| XMRV Isolation Control (IsoC)^a | 0.4 mL |
| XMRV Positive Control (PosC)^b | 0.1 mL |
| XMRV Negative Control (NegC) | 1.25 mL |
| Norgen's DNA Marker | 0.1 mL |
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^a The positive control is an in vitro transcribed XMRV RNA fragments.

^b The isolation control is a cloned PCR product

Customer-Supplied Reagents and Equipment

- Disposable Latex gloves
- Centrifuge with a swinging bucket rotor capable of 2000 x g
- Benchtop microcentrifuge
- Micropipettes with an accuracy range between 1-10 µL, 10-100 µL and 100-1000 µL
- Laminar flow hood for extractions
- Vortex
- Sterile, nuclease-free aerosol-barrier micropipettor tips
- Microcentrifuge tube rack
- PCR tubes
- 96 – 100% ethanol
- β-mercaptoethanol
- 50 mL conical tubes

Storage Conditions and Product Stability

- The Positive Control (*XMRV PosC*, red cap) and Isolation Control (*XMRV IsoC*, orange cap) should be stored at -70 °C. If needed, make aliquots of the controls according to the volume used in the protocol (10 µL of *XMRV PosC* or 15 µL of *XMRV IsoC*) prior to freezing.
- The XMRV 2X RT-PCR Mastermix should be stored at -20 °C. Make appropriate aliquots if needed
- All other kit components may be stored at room temperature
- The XMRV 2X RT-PCR Mastermix, XMRV Positive Control (PosC) and XMRV Isolation Control (IsoC) should not undergo repeated freeze-thaw (a maximum freeze-thaw of three times).
- Allow reagents to thaw at room temperature prior to use
- After addition of samples to RT-PCR Master Mix use within one hour

General Precautions

- Follow universal precautions. All patient specimens should be considered as potentially infectious and handled accordingly.
- Diagnostic laboratory work on clinical samples from patients who are suspected of having XMRV infection should be conducted in a BSL2 laboratory. All sample manipulations should be carried out in a biosafety cabinet. Viral isolation on clinical specimens from patients who are suspected of having XMRV infection should be performed in a BSL2 laboratory with BSL3 practices
- Wear personal protective equipment, including gloves and lab coats when handling kit reagents. Wash hands thoroughly when finished performing the test.
- Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
- Dispose of unused kit reagents and human specimens according to local, provincial or federal regulations.
- Workflow in the laboratory should proceed in a uni-directional manner, beginning in the pre-amplification area(s) (i.e. specimen collection and RNA extraction) and moving to the amplification / detection area(s) (RT-PCR and gel electrophoresis).
- Do not use supplies and equipment across the dedicated areas of specimen extraction and sample preparation. No cross-movement should be allowed between the different areas.
- Supplies and equipment used for specimen preparation should not be used for pipetting or processing amplified RNA or other sources of target nucleic acids.
- All amplification supplies and equipment should be kept in the amplification / detection area at all times.
- Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- As contamination of patient specimens or reagents can produce erroneous results, it is essential to use aseptic techniques.
- Pipette and handle reagents carefully to avoid mixing of the samples.
- Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- Do not substitute or mix reagents from different kit lots or from other manufacturers.
- Do not interchange reagent tube / bottle caps as this may lead to contamination and compromise test results.
- Only use the protocol provided in this insert. Alterations to the protocol and deviations from the times and temperatures specified may lead to erroneous results.

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's Urine-Based XMRV RT-PCR Detection Kit, the XMRV 2x RT-PCR Master Mix, the *XMRV Isolation Control (IsoC)*, the *XMRV Negative Control (NegC)* and the *XMRV Positive Control (PosC)* are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's Urine-Based XMRV RT-PCR Detection Kit is designed for research purposes only. It is not intended for human or diagnostic use.

Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Safety Information

Biosafety level 2 practices are recommended for works involving clinical samples from patients who are suspected of having XMRV infection. Ensure the appropriate containment equipment and facilities are used for activities involving cultures or potentially infectious clinical materials. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at www.norgenbiotek.com.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

The **Lysis Solution** contains guanidine salts, and should be handled with care. Guanidine salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

1. Protocol

A. Specimen Collection, Storage and Transport

Precaution: All samples have to be treated as potentially infectious material.

1. Specimen Collection and Sample Storage

- Midstream urine samples should be collected, as the first flow of urine has been shown to have a higher rate of contamination (Morimoto *et al.*, 2003).
- It is highly recommended that urine samples be collected using Norgen's Urine Collection and Preservation Tubes (Cat# 18111). The urine samples can be stored for at least one year at room temperature when collected directly using Norgen's Urine Collection and Preservation Tubes.
- Alternatively, urine samples collected using any other collection and preservation systems or reagents are also compatible with this kit.

2. Sample Transport

- Sample material should be transported in a shatterproof, leak-proof transport container as a matter of principle. Thus, a potential danger of infection due to a leakage of sample can be avoided.
- The samples should be transported following the local and national instructions for the transport of pathogen material.

B. Isolation of RNA from Urine

Notes:

- Do not spin down or filter the urine sample before proceeding with the isolation, as this could negatively affect the isolation of XMRV viral RNA.
 - All centrifugation steps are performed at room temperature.
 - A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
 - Ensure that all solutions are at room temperature prior to use.
 - Prepare a working concentration of the RNA Wash Solution by adding 66 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated RNA Wash Solution. This will give a final volume of 90 mL. The bottle label contains a box to check to indicate that the ethanol has been added.
 - **Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of RNA Lysis Solution required.** β-mercaptoethanol is toxic and should be dispensed in a fume hood.
 - If precipitates are present in the RNA Lysis Solution it is highly recommended to warm up the RNA Lysis Solution at 60°C for 20 minutes and mix well until the solution becomes clear again.
 - It is important to work quickly during this procedure.
 - Elevated levels of bilirubin (≥15 mg/dl) and lipids (≥800 mg/dl) and haemolytic samples do not influence the system
 - An *XMRV Isolation Control (IsoC)* is supplied. This allows the user to control the viral RNA isolation procedure. For this assay, add the *XMRV Isolation Control (IsoC)* to the lysate during the isolation procedure.
 - The *XMRV Isolation Control (IsoC)* **must not** be added to the sample material directly
 - Do not freeze and thaw the *XMRV Isolation Control (IsoC)* more than 3 times.
 - The *XMRV Isolation Control (IsoC)* must be kept on ice at all times during the isolation procedure.
1. Obtain a 10 mL midstream urine sample. Add 10 mL of **RNA Lysis Solution** directly to the urine. Lyse cells by **vortexing** for 15 seconds. (**Note: RNA Lysis Solution contains resin and must be mixed well before every pipeting**)
 2. Add 5 mL of 95 - 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds
 3. Add 15 µL of *XMRV Isolation Control (IsoC)* to the lysate. **Vortex for 10 seconds.**
 4. Centrifuge for **5 minutes at 2,000 x g**, Discard the supernatant.
 5. Add 500 µL **RNA Wash Solution**, mix well by pipeting and then transfer the entire contents into a Mini Filter Spin column and centrifuge for **1 minute at 14,000 x g (~14,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
 6. Apply 500 µL of **RNA Wash Solution** to the column and centrifuge for **1 minute at 14,000 x g (~14,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.
 7. Repeat Step 6.
 8. Spin the column, empty, for **3 minutes at 14,000 x g (~14,000 RPM)**. Discard the collection tube.
 9. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply 100 µL of **RNA Elution Solution** to the column and centrifuge for **2 minutes at 200 x g (~2,000 RPM)**, followed by **3 minute at 14,000 x g (~14,000 RPM)**.

C. XMRV RT-PCR Assay Preparation

Notes:

- It is recommended that 10 μL of the RNA elution be used as the RT-PCR sample input volume
 - Sample volume can be varied between 2 μL – 10 μL of the RNA elution. PCR grade water should be added to make up the final volume of the RT-PCR reaction to 20 μL .
 - Using a lower volume from the sample than recommended may affect the sensitivity of the XMRV Limit of Detection.
 - *XMRV Negative Control (NegC)* and *XMRV Positive Control (PosC)* must be included during every run.
 - The *XMRV Negative Control (NegC)* and *XMRV Positive Control (PosC)* provided are sufficient for eight PCR runs.
 - Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or quick vortexing), and centrifuged briefly.
 - The XMRV 2X RT-PCR Master Mix and *XMRV Positive Control (PosC)* should be kept on ice during PCR set-up.
1. Prepare RT-PCR reactions as outlined in Table 1 below. For each sample to be run, pipette 10 μL of the eluted RNA and 10 μL of the Master Mix into a PCR tube. Each RT-PCR reaction will have a final volume of 20 μL .
 2. *XMRV Negative Control (NegC)* and *XMRV Positive Control (PosC)* must be included in every run. Pipette 10 μL of *XMRV Negative Control (NegC)* into a PCR tube and add 10 μL of Master Mix. Pipette 10 μL of *XMRV Positive Control (PosC)* into a PCR tube and add 10 μL of Master Mix.
 3. Program the PCR machine according to the program shown in Table 2 below.
 4. Run RT-PCR.

Table 1. PCR Assay Preparation

| Preparation of RT-PCR assay | Volume Per RT-PCR Reaction | | |
|-------------------------------------|----------------------------|------------------|------------------|
| XMRV 2X RT-PCR Master Mix | 10 μL | 10 μL | 10 μL |
| Sample (Eluted RNA) | 10 μL | | |
| <i>XMRV Positive Control (PosC)</i> | ----- | 10 μL | ----- |
| <i>XMRV Negative Control (NegC)</i> | ----- | ----- | 10 μL |
| Total Volume | 20 μL | 20 μL | 20 μL |

Table 2. XMRV RT-PCR Assay Program

| PCR Cycle | Step | Temperature | Duration |
|----------------------|--------|-------------|----------|
| Cycle 1 | Step 1 | 50°C | 30 min |
| Cycle 2 | Step 1 | 95°C | 3 min |
| Cycle 3 (40x) | Step 1 | 94°C | 15 sec |
| | Step 2 | 64°C | 30 sec |
| | Step 3 | 72°C | 45 sec |
| Cycle 4 | Step 1 | 72°C | 5 min |
| Cycle 5 | Step 1 | 4°C | ∞ |

D. XMRV PCR Assay Interpretation

- For the analysis of the PCR data, the entire 20 µL PCR reaction should be loaded on a 1X TAE, 2% Agarose DNA gel along with 10 µL of Norgen's DNA Marker (provided).
- The PCR products should be resolved on the 1X TAE, 2% Agarose gel at 150V for 30 minutes
- Figure 1 and Table 4 explain how to interpret the PCR assay results

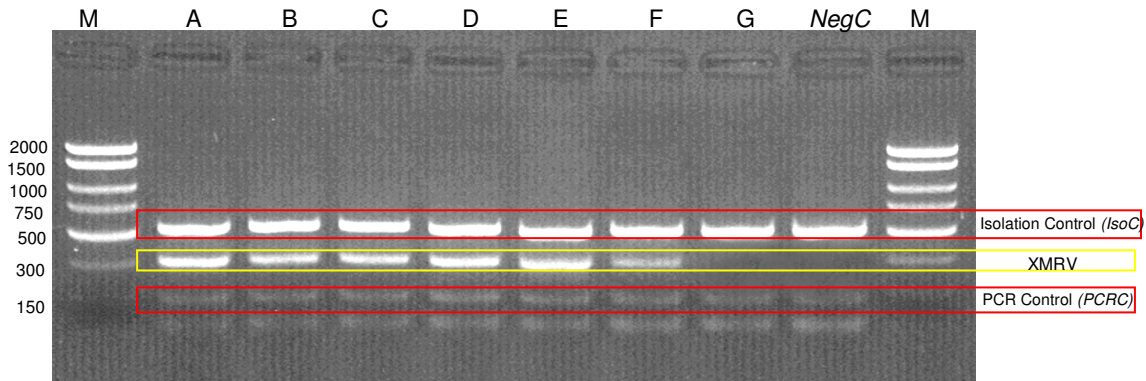


Figure 1: A representative 1X TAE, 1.7% agarose gel showing the amplification of XMRV at different concentrations (*Target*). The size of the XMRV target amplicon corresponds to the 300 bp band represented by the provided DNA Marker (M). The size of the *XMRV Isolation Control (IsoC)* corresponds to the 500bp band represented by the provided DNA Marker (M). The XMRV 2X RT-PCR Master Mix contains a XMRV PCR Control (*PCRC*). The XMRV PCRC Controls for PCR inhibition. The size of the XMRV PCRC corresponds to the 150bp band represented by the provided DNA Marker (M). Lanes A-H represents samples spiked with different XMRV concentrations isolated from 10mL urine samples (interpreted as positive results). The XMRV spiked in urine samples is an in vitro transcribed XMRV RNA fragments.

Table 3. Interpretation of RT-PCR Assay Results

| Input Type | XMRV IsoC Band (500 bp) | XMRV Target Band (300 bp) | XMRV PCRC Band (150 bp) | Interpretation |
|------------------|-------------------------|---------------------------|-------------------------|----------------|
| Positive Control | X | X | X | Valid |
| Negative Control | | | X | Valid |
| Sample | X | X | X | Positive |
| Sample | X | | X | Negative |
| Sample | | X | X | Positive |
| Sample | X | X | | Positive |
| Sample | | X | | Positive |

** For results obtained that are not covered in Table 3 above, please refer to the Troubleshooting Section.

E. Specificity

The specificity of Norgen's Urine-Based XMRV RT-PCR Detection Kit is first and foremost ensured by the selection of the XMRV-specific primers, as well as the selection of stringent reaction conditions. The primers were checked for possible homologies in GenBank published sequences by sequence comparison analyses. Furthermore, the specificity of the XMRV-specific primers were tested against most of the known sexually-transmitted pathogens.

F. Linear Range

- The linear range of Norgen's Urine-Based XMRV RT-PCR Detection Kit was determined by analyzing a dilution series of XMRV quantitative standard ranging from 8.46×10^9 VP/ μ l to 1×10^{-1} IU/ μ l.
- Each dilution has been tested in replicates ($n = 4$) using Norgen's Urine-Based XMRV RT-PCR Detection Kit on 1X TAE, 1.7% Agarose gels.
- The linear range of Norgen's Urine-Based XMRV RT-PCR Detection Kit has been determined to cover concentrations from 20 VP/ μ l to at least 8×10^5 VP/ μ l
- Under the conditions of Norgen's Urine RNA Isolation procedure, Norgen's Urine-Based XMRV RT-PCR detection Kit covers a linear range from 2000VP/mL urine to at least 8×10^9 VP/mL urine.

G. Frequently Asked Questions

1. How many samples should be included per PCR run?

- Norgen's Urine-Based XMRV RT-PCR Detection Kit is designed to test 24 samples. For every 6 samples, a Negative Control and a Positive Control must be included. It is preferable to pool and test 6 samples at a time. If not, the provided Negative Control and Positive Control are enough to run 3 samples at a time.

2. How can I interpret my results for a sample if neither the XMRV PCR control nor the XMRV Isolation Control (IsoC) amplifies?

- If neither the XMRV PCR control nor the XMRV Isolation Control (IsoC) amplifies, the sample must be re-tested. If the positive control showed amplification, then the problem occurred during the isolation, where as if the Positive control did not amplify the problem has occurred during the setup of the PCR assay reaction.

3. How should it be interpreted if only the XMRV PCR control showed amplification but neither the XMRV target nor the XMRV Isolation Control (IsoC) amplified for a sample?

- This indicates a poor isolation. The isolation procedure must be repeated.

4. How should it be interpreted if only the XMRV Isolation Control (IsoC) was amplified in a sample?

- The sample tested can be considered as XMRV negative.

5. How should it be interpreted if only the XMRV target and the XMRV PCR control were amplified in a sample?

- The sample tested can be considered as XMRV positive.

6. How should it be interpreted if only the XMRV target was amplified in a sample?

- The sample tested can be considered positive. At high XMRV viral load, the XMRV amplicon will be predominant and the XMRV PCR control as well as the XMRV Isolation control may not amplify.

7. How should it be interpreted if only the XMRV PCR control and the XMRV Isolation Control (IsoC) showed amplification?

- The sample tested can be considered negative

8. Can I process a different urine volume?

- The reagents provided with the isolation kit are only sufficient to process 24 urine samples of 5mL each.

9. What If I added more or less of the specified reagents' volume during RNA isolation?

- Adding less volume may reduce your RNA yields. Adding more may not affect the RNA yields EXCEPT if more Elution Buffer was added. Eluting RNA in higher volumes of Elution Buffer will result in diluting your RNA.

10. What If I forgot to do a dry spin after my second wash?

- Your RNA elution will be contaminated with the Wash Solution. This may dilute the RNA yield in your elution and it may interfere with your down stream applications.

11. What If I forgot to add the XMRV Isolation control during the Isolation?

- The Isolation must be repeated.

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Urine-based XMRV PCR Detection Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362 or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

References

Morimoto, M., Yanai, H., Chiba, H., Matsuno, K. and Shukuya, K. (2003). Importance of midstream clean-catch technique for urinalysis, reconfirmed by urinary flow cytometry. *Clin Chim Acta*. 333, 101-102.

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