Toxoplasma gondii PCR Detection Kit
Product # 44700

Pathogen Information
Toxoplasma gondii is a single-celled organism which causes Toxoplasmosis, the most common parasitic infection worldwide affecting animals as well as humans. Cats are the only animal in which sexual reproduction of the organism occurs, and for this reason cats are the only domestic animal which has the potential to shed T. gondii eggs. When disease does occur, it may develop following primary infection due to an inadequate immune response to stop the invasive tachyzoites or as a result of a reactivated infection due to a compromised immune system. The most common symptoms of the disease are anorexia, weight loss, lethargy, dyspnea, ocular symptoms and pyrexia. As toxoplasmosis can be transmitted to humans it represents a serious health risk for people living in close contact with infected animals. Infection is especially dangerous for people with suppressed immune systems and cancer patients as well as pregnant women.

Principle of the Test
Norgen’s Toxoplasma gondii PCR Detection Kit constitutes a ready-to-use system for the isolation and detection of Toxoplasma gondii using end-point PCR. The kit first allows for the isolation of Toxoplasma gondii DNA from the blood samples using spin-column chromatography. The Toxoplasma gondii DNA is isolated free from inhibitors, and can then be used as the template in a PCR reaction for Toxoplasma gondii detection using the provided Toxoplasma gondii Master Mix. The Toxoplasma gondii Mastermix contains reagents and enzymes for the specific amplification of a 303 region of the genome. In addition, Norgen’s Toxoplasma gondii PCR Detection Kit contains a second Mastermix, the PCR Control Master Mix, which can be used to identify possible PCR inhibition and/or inadequate isolation via a separate PCR reaction with the use of the provided PCR control (PCRC) or Isolation Control (IsoC), respectively. This kit is designed to allow for the testing of 24 samples.

Kit Components:

<table>
<thead>
<tr>
<th>Component</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis Solution</td>
<td>18 mL</td>
</tr>
<tr>
<td>Wash Solution</td>
<td>12 mL</td>
</tr>
<tr>
<td>Elution Buffer</td>
<td>6 mL</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>0.6 mL</td>
</tr>
<tr>
<td>Spin Columns inserted in Collection Tubes</td>
<td>25</td>
</tr>
<tr>
<td>Collection Tubes</td>
<td>25</td>
</tr>
<tr>
<td>Elution tubes (1.7 mL)</td>
<td>25</td>
</tr>
<tr>
<td>TOX 2x Detection PCR Master Mix</td>
<td>0.35 mL</td>
</tr>
<tr>
<td>Control 2x PCR Master Mix</td>
<td>0.35 mL</td>
</tr>
<tr>
<td>Isolation Control (IsoC)*</td>
<td>0.3 mL</td>
</tr>
<tr>
<td>TOX Positive Control (PosC)*</td>
<td>0.1 mL</td>
</tr>
<tr>
<td>Nuclease Free-Water</td>
<td>1.25 mL</td>
</tr>
<tr>
<td>Norgen’s DNA Marker</td>
<td>0.1 mL</td>
</tr>
<tr>
<td>Product Insert</td>
<td>1</td>
</tr>
</tbody>
</table>

*a IsoC = Isolation Control ; PosC= Positive Control
*b The isolation control is a cloned PCR product.
*b The positive control is a fragment of Toxoplasma cloned in a plasmid
Customer-Supplied Reagents and Equipment
- Benchtop microcentrifuge
- 1.5 mL microcentrifuge tubes
- 96 – 100% ethanol
- Isopropanol
- 55°C water bath or incubator

Storage Conditions and Product Stability
All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers. The kit contains a ready-to-use Proteinase K solution, which is dissolved in a specially prepared storage buffer. The Proteinase K is stable for up to 1 year after delivery when stored at room temperature. To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

The TOX 2x PCR Master Mix, Control 2x PCR Master Mix, TOX Positive Control (PosC) and the Isolation Control (IsoC) should be kept tightly sealed and stored at -20°C for up to 1 year without showing any reduction in performance. Repeated thawing and freezing (> 2 x) should be avoided, as this may reduce the sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots.

General Precautions
The user should exercise the following precautions when using the kit:
- Use sterile pipette tips with filters.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice.

Quality Control
In accordance with Norgen’s ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen’s *Toxoplasma gondii* PCR Detection Kit, including the TOX 2x PCR Master Mix, Control 2x PCR Master Mix, Isolation Control and TOX Positive Control are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations
Norgen’s *Toxoplasma gondii* PCR Detection Kit is designed for research purposes only.

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.

Precautions and Disclaimers
This kit is designed for research purposes only. It is not intended for human or diagnostic use.

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

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.
Blood of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with blood.

**Safety Information**

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](http://www.norgenbiotek.com).

**Important Notes Prior to Beginning Protocol:**

- Blood of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood.
- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed microcentrifuge 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, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- For best results, the use of whole blood collected into tubes containing an anticoagulant is highly recommended.
- Both fresh and frozen anticoagulated blood may be used with this procedure. Ensure that frozen blood is thawed at room temperature prior to starting the protocol.
- Prepare a working concentration of Wash Solution by adding 28 mL of 96 – 100 % ethanol (provided by the user) to the supplied bottle containing concentrated Wash Solution. This will give a final volume of 40 mL. The label on the bottle has a box that can be checked to indicate that ethanol has been added.
- Always vortex the Proteinase K before use.
- **Isolation Control (IsoC)**
  - An Isolation Control (IsoC) is supplied. This allows the user to control the DNA isolation procedure. For this assay, add the Isolation Control (IsoC) to the lysate during the isolation procedure.
  - The Isolation Control (IsoC) must not be added to the sample material directly.
  - Do not freeze and thaw the Isolation Control (IsoC) more than 2 times.
  - The Isolation Control (IsoC) must be kept on ice at all times during the isolation procedure.
- The PCR components of the Toxoplasma gondii PCR Detection Kit should remain at -20°C until DNA is extracted and ready for PCR amplification.
- It is important to work quickly during this procedure.

**A. Lysate Preparation**

1. Add 12 µL of **Proteinase K** to a microcentrifuge tube.
2. Transfer up to 500µL of blood sample to the tube containing Proteinase K.
3. Add 600 µL of **Lysis Solution** to the blood and mix well by gentle vortexing for 10 seconds.
4. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
5. Incubate at 55°C for 10 minutes.
6. If any debris is present in the sample, centrifuge for 2 minutes at 14,000 x g (~14,000 RPM) to precipitate. Transfer the clean supernatant to a microcentrifuge tube prior to **Step 7**.
7. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
8. Add 240 µL of Isopropanol to the sample and mix well by gentle vortexing for 10 seconds.
9. Briefly spin the tube to collect any drops of liquid from the inside of the lid.
B. Specimen DNA Purification
Following the lysate preparation, DNA can be extracted from the patient specimens using the supplied buffers and solutions according to the following protocol:

1. Add 10 µL of Isolation Control (IsoC) to the lysate mixture and vortex briefly.
2. Obtain a spin column assembled with its collection tube. Apply up to 650 µL of the lysate mixture to the column and centrifuge for 1 minute at 6,000 x g (~8,000 RPM).
3. Discard the flowthrough. Reassemble the column and the collection tube.

   **Note:** Ensure that all of the lysate has passed through into the collection tube. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

4. Repeat step B2 and B3 with the remaining lysate
5. Discard the collection tube containing flow-through.
6. Assemble a spin column with a new collection tube.
7. Apply 500 µL of Wash Solution (ensure ethanol was added) to the column and centrifuge for 1 minute at 6,000 x g (~8,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.

   **Note:** Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

8. Wash column a second time by adding 500 µL of Wash Solution and centrifuging for 1 minute at 6,000 x g (~8,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.
9. Spin the column for 2 minutes in order to thoroughly dry the resin at 14,000 x g (~14,000 RPM). Discard the collection tube.
10. Place the column into a provided 1.7 mL elution tube.
11. Add 200 µL of Elution Buffer to the column.
12. Centrifuge for 1 minute at 6,000 x g (~8,000 RPM).

B. Toxoplasma PCR Assay Preparation

**Notes:**

- Before use, suitable amounts of all PCR components should be completely thawed at room temperature, vortexed and centrifuged briefly.
- The amount of TOX 2X Detection PCR Master Mix and Control 2X PCR Master Mix provided is enough for up to 32 PCR reactions (24 sample PCR, 4 positive control PCR and 4 no template control PCR).
- For each sample, one PCR reaction using the TOX 2X Detection PCR Mastermix and one PCR reaction using Control 2X PCR Mastermix should be set up in order to have a proper interpretation of the results.
- For every PCR run, one reaction containing TOX Positive Control and one reaction as no template control must be included for proper interpretation of results.
- The recommended minimum number of DNA samples tested per PCR run is 6.
- Using a lower volume from the sample than recommended may affect the sensitivity of TOX Limit of Detection.

1. Prepare the PCR reaction for sample detection (Set #1, using **TOX 2X Detection PCR Mastermix**) and the PCR reaction for control detection (Set #2, using **Control 2X PCR Mastermix**) as shown in Table 1 below. The recommended amount of sample DNA to be used is 2.5 µL. However, a volume between 1 and 5 µL of sample DNA may be used as template. Ensure that one TOX detection reaction and one control reaction is prepared for each DNA sample. Adjust the final volume of the PCR reaction to 20 µL using the Nuclease-Free Water provided.
Table 1. PCR Assay Preparation

<table>
<thead>
<tr>
<th>PCR Components</th>
<th>Volume Per PCR Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOX 2X PCR Master Mix Or Control 2X PCR Master Mix</td>
<td>10 µL</td>
</tr>
<tr>
<td>Sample DNA</td>
<td>2.5 µL</td>
</tr>
<tr>
<td>Nuclease-Free Water</td>
<td>7.5 µL</td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td><strong>20 µL</strong></td>
</tr>
</tbody>
</table>

2. For each PCR set, prepare one positive control PCR as shown in Table 2 below:

Table 2. PCR Positive Control Preparation

<table>
<thead>
<tr>
<th>PCR Components</th>
<th>Volume Per PCR Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOX 2X PCR Master Mix Or Control 2X PCR Master Mix</td>
<td>10 µL</td>
</tr>
<tr>
<td><strong>TOX Positive Control (PosC)</strong></td>
<td><strong>10 µL</strong></td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td><strong>20 µL</strong></td>
</tr>
</tbody>
</table>

3. For each PCR set, prepare one no template control PCR as shown in Table 3 below:

Table 3. PCR Negative Control Preparation

<table>
<thead>
<tr>
<th>PCR Components</th>
<th>Volume Per PCR Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOX 2X PCR Master Mix Or Control 2X PCR Master Mix</td>
<td>10 µL</td>
</tr>
<tr>
<td><strong>Nuclease-Free Water</strong></td>
<td><strong>10 µL</strong></td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td><strong>20 µL</strong></td>
</tr>
</tbody>
</table>

C. Toxoplasma PCR Assay Programming

1. Program the thermocycler according to the program shown in Table 4 below.
2. Run one step PCR.
Table 4. Toxoplasma Assay Program

<table>
<thead>
<tr>
<th>PCR Cycle</th>
<th>Step</th>
<th>Temperature</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cycle 1</strong></td>
<td>Step 1</td>
<td>95°C</td>
<td>3 min</td>
</tr>
<tr>
<td><strong>Cycle 2 (35x)</strong></td>
<td>Step 1</td>
<td>94°C</td>
<td>15 sec</td>
</tr>
<tr>
<td></td>
<td>Step 2</td>
<td>60°C</td>
<td>15 sec</td>
</tr>
<tr>
<td></td>
<td>Step 3</td>
<td>72°C</td>
<td>30 sec</td>
</tr>
<tr>
<td><strong>Cycle 3</strong></td>
<td>Step 1</td>
<td>72°C</td>
<td>5 min</td>
</tr>
<tr>
<td><strong>Cycle 4</strong></td>
<td>Step 1</td>
<td>4°C</td>
<td>∞</td>
</tr>
</tbody>
</table>

D. Toxoplasma PCR Assay Results Interpretation

1. For the analysis of the PCR data, the entire 15-20 µL PCR Reaction should be loaded on a 1X TAE 1.7% Agarose DNA gel along with 10 µL of Norgen’s DNA Marker (provided).
2. The PCR products should be resolved on the 1X TAE 1.5% Agarose gel at 150V for 30 minutes (Gel running time will be vary depending on an electrophoresis apparatus).
3. Sample results are provided below:

![Figure 1](image_url)

**Figure 1**: A representative 1X TAE 1.5% agarose gel showing the amplification of TOXOFILARIA at different concentrations (TOXOFILARIA Target). The size of the TOXOFILARIA target amplicon corresponds to 276 bp as represented by the provided DNA Marker (M). **NTC** = Negative Control.
Figure 2: A representative 1X TAE 1.7% agarose gel showing the amplification of Isolation Control and PCR Control under different conditions using the Control 2X PCR Mastermix. The size of the Isolation Control amplicon and PCR Control amplicon correspond to 499 bp and 150 bp, respectively, as represented by the provided DNA Marker (M). Lanes 1 to 5 showed detection of both Isolation Control and PCR Control, suggesting that the DNA isolation as well as the PCR reaction was successful. Lane 6 showed only the detection of PCR Control suggesting that while the PCR was successful, the isolation failed to recover even the spiked-in Isolation control. NTC = Negative Control.

Table 5. Interpretation of PCR Assay Results

<table>
<thead>
<tr>
<th>Input Type</th>
<th>Target reaction</th>
<th>Control Reaction</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Toxoplasma</td>
<td>IsoC Band</td>
<td>PCRC Band</td>
</tr>
<tr>
<td></td>
<td>Target Band</td>
<td>(499 bp)</td>
<td>(150 bp)</td>
</tr>
<tr>
<td>Positive Control</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Negative Control</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Sample</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Sample</td>
<td>X</td>
<td>X</td>
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<td>Sample</td>
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<tr>
<td>Sample</td>
<td>X</td>
<td></td>
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</tbody>
</table>

** For results obtained that are not covered in Table 5 above, please refer to the Troubleshooting Section.
E. *Toxoplasma* PCR Assay Specificity and Sensitivity

- The specificity of Norgen’s *Toxoplasma gondii* PCR Detection Kit is first and foremost ensured by the selection of the *Toxoplasma gondii* specific primers, as well as the selection of stringent reaction conditions. The *Toxoplasma gondii* specific primers were checked for possible homologies to GenBank published sequences by sequence comparison analysis and published strains.

F. Linear Range

- The linear range of Norgen’s *Toxoplasma gondii* PCR Detection Kit was determined by analysing a dilution series of a *Toxoplasma gondii* quantification standards ranging from 100 ag to 1 pg.
- Each dilution has been tested in replicates (n = 4) using Norgen’s *Toxoplasma gondii* PCR Detection Kit on a 1X TAE 1.7% agarose gel.
- The linear range of Norgen’s *Toxoplasma gondii* PCR Detection Kit has been determined to cover concentrations from 100 ag to 1 ng.
- Under the conditions of the Norgen’s *Toxoplasma gondii* DNA Isolation procedure, Norgen’s *Toxoplasma gondii* PCR Detection Kit covers a linear range from 100 copies to $1 \times 10^6$ copies.

Frequently Asked Questions

1. **How many samples should be included per PCR run?**
   - Norgen’s *Toxoplasma gondii* PCR Detection Kit is designed to test 24 samples. For every 6 samples, a non-template control (Nuclease Free Water) and a Positive Control must be included. It is preferable to pool and test 6 samples at a time. If not, the provided Positive Control is enough to run 3 samples at a time.

2. **How can I interpret my results if neither the TOX PCR control nor the TOX Isolation Control (IsoC) amplifies?**
   - If neither the TOX PCR control nor the TOX 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, therefore the problem has occurred during the setup of the PCR assay reaction.

3. **How should it be interpreted if only the TOX PCR control showed amplification but neither the TOX target nor the TOX Isolation control amplified for a sample?**
   - This indicates a poor isolation. The isolation procedure must be repeated.

4. **How should it be interpreted if only the TOX Isolation Control (IsoC) was amplified in a sample?**
   - The sample tested can be considered as *Toxoplasma gondii* negative.

5. **How should it be interpreted if the TOX PCR control and the TOX target showed amplification in a sample?**
   - The sample tested can be considered positive. It could happen when too much template was added to the reaction.

6. **How should it be interpreted if only the TOX target and the TOX PCR control were amplified in a sample?**
   - The sample tested can be considered as *Toxoplasma gondii* positive.

7. **How should it be interpreted if only the TOX target was amplified in a sample?**
   - It is recommended that the isolation is repeated.
8. How should it be interpreted if only the *TOX* PCR control and the *TOX* Isolation control showed amplification in a sample?
   - The sample tested can be considered negative

9. What if I forgot to do a dry spin after my third wash?
   - Your first DNA elution will be contaminated with the Wash Solution. This may dilute the DNA yield in your first elution and it may interfere with the PCR detection, as ethanol is known to be a PCR inhibitor.

10. What if I forgot to add the *TOX* Isolation Control (*IsoC*) during the isolation?
    - It is recommended that the isolation is repeated.

11. What if I forgot to run the Control RT-PCR for the sample and I only ran the Detection RT-PCR and I obtained a positive result?
    - The result can be considered positive. However, any negative result must be verified by running the associated control PCR to ensure that it is a true negative and not a false negative due to problems with the RNA isolation or the PCR reactions.

<table>
<thead>
<tr>
<th>Related Products</th>
<th>Product #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Genomic DNA Isolation Mini Kit</td>
<td>31000</td>
</tr>
<tr>
<td>Blood DNA Preservation Buffer (3X)</td>
<td>29111</td>
</tr>
<tr>
<td><em>Dirofilaria immitis</em> PCR Detection Kit</td>
<td>44500</td>
</tr>
<tr>
<td><em>Leptospira interrogans</em> PCR Detection Kit</td>
<td>44600</td>
</tr>
</tbody>
</table>

**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 *Toxoplasma gondii* 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](http://www.norgenbiotek.com)) or through email at techsupport@norgenbiotek.com.