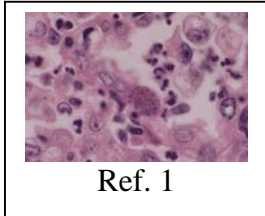


Toxoplasma gondii Detection with real time PCR reagents

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Overview:



Toxoplasma gondii is a common intestinal parasite of cats. Accidental infection of humans may cause disease ranging from asymptomatic to pharyngitis with cervical adenopathy, fever and myalgia, systemic disease of the eye, heart, muscle liver or brain. These may be particularly severe in the immune compromised. Infection of pregnant woman may cause severe congenital infection.

Primers and probe are available for amplification and detection of the T. gondii. The T. gondii primers are specific for a 97 bp portion of a DNA repeat sequence. The T. gondii detection probe is a molecular beacon. (2)

Products

| Products | Catalog No. | Quantity |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|-----------------|
| T. gondii FAM-BHQ1 Primer-probe <i>(Store at -20C.) 25-20 µl reactions</i> <i>Typical use: Add 2X master mix and DNA, then thermal cycle.</i> <i>Attostar reagent contains primers and probe. Detection at 510nm.</i> | PP5000 | 0.055ml |
| AttoMaster 2X Mix for qPCR <i>Store at -20°C. 125-20 ul reactions</i> <i>Contains Taq polymerase (requires heat activation), dNTPs (0.4 mM) with optimal dUTP to dTTP ratio, heat labile UDG, Mg(6 mM), and buffer.</i> <i>Typical use: Add Attostar Primer-probe, DNA, and then thermal cycle.</i> | AM10 | 1.25 ml |
| T. gondii Plasmid <i>(Store at -20C.)</i> <i>Typical use: make serial 10 fold dilutions in TE for standard curve, diluting 5 ul into 45 ul TE.</i> | PLAS5000 | 0.25ml |

Detection of Toxoplasma DNA:

.....
Thermal cycle conditions for PCR reactions on RotorGene*

*Similar cycle conditions and reaction volumes may be used on many other thermal cyclers.

- 25°C 10 min (UDG treatment time)
- 95°C 120 sec (activation for AttoMaster polymerase)
- 40 cycles
 - 95°C 15 seconds
 - 60°C 30 seconds RotorGene Channel Setup FAM/Sybr, Cy5; Gain 7
 - 72°C 30 seconds

FAM/Sybr has a source of 470nm and Detector 510nm (LightCycler use F1)
Cy5 has a source of 625nm and Detector 660hp nm
Quasar 670 has the same fluorescent absorption and emission as Cy5.

.....
Thermal cycle conditions for PCR reactions on LightCycler

- 25°C 10 min (UDG treatment time)
- 95°C 120 sec (activation for AttoMaster polymerase)
- 40 cycles
 - 95°C 15 seconds
 - 60°C 30 seconds acquire fluorescent signal on F1 gain =1
 - 72°C 30 seconds
 - 40°C 30 seconds cool

.....
Use of the Toxoplasma gondii plasmid:

Dilute the plasmid in TE to prepare a standard curve. Common dilutions would be 10-fold from 200 to 0.002pg/ml. The 0.02 pg/ml plasmid dilution contains 10 copies of plasmid in 2 µl.

.....
Attostar Primers/probe PP5000 RotorGene-PCR 20µl reactions

| RotorGene Reagents needed for 20 ul PCR final reaction tube volumes | | | | | | | | | | |
|---------------------------------------------------------------------|----|----|----|----|----|----|----|----|----|--------|
| Reaction tube number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Attostar Primer-Probe FAM labeled (10X) | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 µl |
| Master mix (2X) | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 µl |
| Dispense 12 ul / reaction tube | | | | | | | | | | |
| Add 8 ul DNA / reaction tube | | | | | | | | | | |

Attostar Primers/probe PP5000 LightCycler-PCR 20 µl reactions

| LightCycler Reagents needed for 20 ul PCR final reaction tube volumes | | | | | | | | | | |
|-----------------------------------------------------------------------|----|----|----|----|----|----|----|----|----|--------|
| Reaction tube number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Attostar Primer-Probe (10X) | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 µl |
| Master mix (2X) | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 µl |
| BSA 1 mg/ml | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 µl |
| Dispense 13 ul / reaction tube | | | | | | | | | | |
| Add 7 ul DNA / reaction tube | | | | | | | | | | |

.....

Extraction / amplification control with T4 bacteriophage

Adding T4 bacteriophage (BAC130) to the sample provides DNA for extraction, amplification, and reaction condition PCR controls.

When added to a sample, T4 adds a known amount of DNA. The T4 DNA can then be extracted, amplified, and detected as a control. T4 controls for the efficiency of DNA extraction, the presence of PCR amplification inhibitors, intact amplification reagents (DNA polymerase, buffer, dNTPs), and instrument function (thermal cycling and fluorescent detection system).

The T4 DNA may be detected in a separate PCR reaction (using FAM labeled T4 probe in PP100). Or the T4 DNA and test organism DNA may be detected using a multiplex reaction (using Quasar 670 labeled T4 probe in PP160 and FAM labeled test organism probe).

Brief procedure for use of T4 as extraction and amplification control:

- Add 5µl T4 bacteriophage to the sample. Proceed with DNA extraction. Dilutions of the bacteriophage may be made to give a final PCR Ct value that is about 35. At this dilution, the phage is more sensitive, i.e. more likely, to detect a poor extraction or the presence of PCR inhibitors in the reaction.

Please refer to the BAC130, PP100, and PP160 product literature.

T4 multiplex reaction (PP160)

RotorGene Multiplex PCR 20µl reactions

| Multiplex Reagents needed for 20 ul PCR final reaction tube volumes | | | | | | | | | | |
|---------------------------------------------------------------------|----|----|----|----|----|----|----|----|----|--------|
| Reaction tube number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Attostar Primer-Probe PP160 | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 µl |
| Primer-Probe mix for TEST organism | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 µl |
| 2 x master mix | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 µl |
| Dispense 14 ul / reaction tube | | | | | | | | | | |
| Add 6 ul DNA / reaction tube | | | | | | | | | | |

| Reaction tube number | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|--------|
| Attostar Primer-Probe PP160 | 22 | 24 | 26 | 28 | 30 | 32 | 34 | 36 | 38 | 40 µl |
| Primer-Probe mix for TEST organism | 22 | 24 | 26 | 28 | 30 | 32 | 34 | 36 | 38 | 40 µl |
| 2 x master mix | 110 | 120 | 130 | 140 | 150 | 160 | 170 | 180 | 190 | 200 µl |
| Dispense 14 ul / reaction tube | | | | | | | | | | |
| Add 6 ul DNA / reaction tube | | | | | | | | | | |

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References:

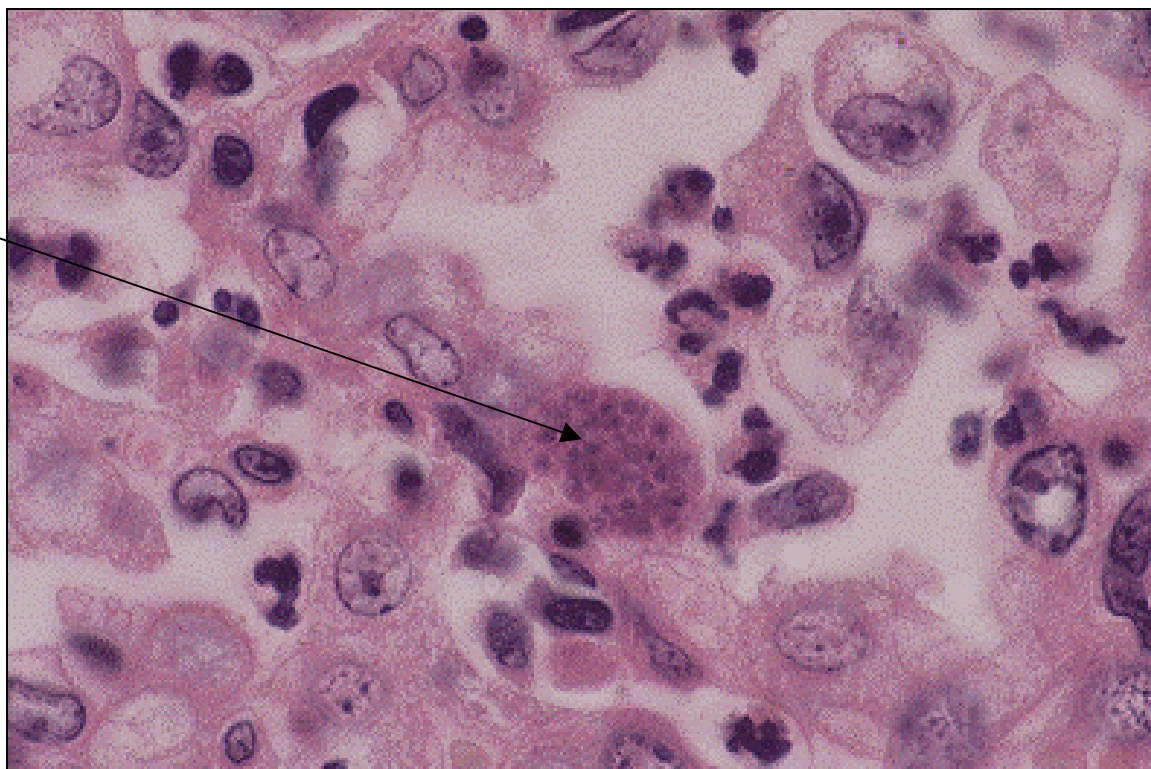
- 1) Image from: <http://www.afip.org/vetpath/WSC/WSC95/95wsc10.htm>
- 2) <http://www.molecular-beacons.org/Introduction.html>

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For technical support, contact Attostar@Attostar.com

Enlarged image (1):

Pneumonia with intracytoplasmic tachyzoites.



10-19-07