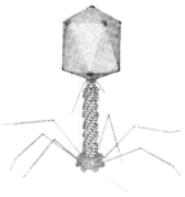
Attostar T4 Bacteriophage
As a DNA extraction and PCR control

Overview:



Bacteriophage T4 was likely isolated by Demerec and Fano (1945) from a phage mixture provided by Dr Tony L Rakieten,(1) The Long Island College of Medicine. The original source is speculated to be from a lysate inoculated with raw sewage.

T4 contains a 168 kb dsDNA genome. 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, the presence of intact amplification reagents (Taq, buffer, dNTPs), and instrument functions (thermal cycling and fluorescence detection).

Primers, probe, and master mix are available for amplification and detection of T4 DNA. The T4 primers are specific for a 163 bp portion of the tail tube glycoprotein 18 gene. The T4 detection probe is a molecular beacon. (2)

Products	**Catalog No.**	**Quantity**

AttoMaster 2X Mix for qPCR **AM10** **1.25 ml**
Store at –20ºC. 125-20 ul reactions
Contains Taq polymerase (requires heat activation), dNTPs (0.4 mM) with optimal dUTP to dTTP ratio, heat labile UDG, Mg(6 mM), and buffer.
Typical use: Add Attostar Primer-probe, DNA, and then thermal cycle.

T4 bacteriophage DNase resistant **BAC130** **0.5 ml**
Store at 4C.
Typical use: add 5 µl (a dilution) of T4 to 100 ul samples prior to DNA extraction. Amplify and detect PCR product to obtain approximate Ct=35.
Dilute with water, not TE.

T4 bacteriophage, 90% DNase sensitive **BAC120** **0.5 ml**
Store at -20C.
Typical use: add 5 µl (a dilution) T4 to 100 ul samples prior to DNA extraction. Amplify and detect PCR product to obtain approximate Ct=35.

T4 bacteriophage Plasmid 200 pg/ml **PLAS100** **0.25ml**
Store at –20C.
Typical use: make serial 10 fold dilutions in TE for standard curve, diluting 5 µl into 45 µl TE buffer.

T4 Master mix Quasar 670-BHQ2** **MM150** **0.175ml**
Store at –20C. 8-24 µl reactions
Typical use: Add DNA polymerase and DNA, then thermal cycle.
Master mix contains buffer, dNTPs, primers, and probe. Detection at 660nm.

T4 Master mix FAM-BHQ1 **MM160** **0.175ml**
Store at –20C. 8-24 µl reactions
Typical use: Add DNA polymerase and DNA, then thermal cycle.
Master mix contains buffer, dNTPs, primers, and probe. Detection at 510nm.

T4 Primers-probe FAM-BHQ1 **PP100** **0.055ml**
Store at –20C. 20-25 µl reactions
Typical use: Add 2X master mix and DNA, then thermal cycle.
Attostar reagent contains primers and probe. Detection at 510nm.

T4 Primers-probe Quasar 670-BHQ2 **PP150** **0.055ml**
Store at –20C. 20-25 µl reactions
Typical use: Add 2X master mix and DNA, then thermal cycle.
Attostar reagent contains primers and probe. Detection at 660nm.

T4 Primers-probe Quasar 670-BHQ2 (multiplex) **PP160** **0.055ml**
Store at –20C. 20-25 µl reactions
Typical use: Add additional primers and probes for
Multiplex reaction. Add 2X master mix and DNA, then thermal cycle.
Attostar reagent contains primers and probe optimized for multiplex reactions.
Detection at 660nm.

T4 Primers-probe Quasar 670-BHQ2 (multiplex) **PP161** **0.2 ml**
Store at –20C. 80-25 µl reactions
Typical use: These primers and probes are a 10X mixture,
similar to PP160, optimized for multiplex reactions.
Add additional primers and probes for
Multiplex reaction. Add 2X master mix (AM10) and DNA, then thermal cycle.
Attostar reagent contains primers and probe optimized for multiplex reactions.
Detection at 660nm.

**Quasar 670 cannot be used on ABI7700 or ABI7000.

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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 functions (thermal cycling and fluorescence detection).

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

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

- Add 5µl 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. If multiplex reactions are being used, this dilution is less likely to compete with the test organism PCR reaction.

Use of the T4 plasmid:

- Dilute the plasmid in TE to prepare a standard curve. Common dilutions would be 10-fold from 200 to 0.02pg/ml. The 0.02 pg/ml plasmid dilution contains 12 copies of plasmid in 2 µl.

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Detection of T4 DNA:

Attostar Master T4 master mixes

These master mixes require the addition of AmpliTaq Gold polymerase. (available from Applied Biosystems, Part number AmpliTaq Gold LD. Obtain from Applied Biosystems. Catalog number 4338857(1000 units) or 438856 (250 units). https://www2.appliedbiosystems.com/

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Thermal cycle conditions for PCR reactions on RotorGene*

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

95C 600 sec (activation for AmpliTaq Gold polymerase)
40 cycles
95C 15 seconds
60C 30 seconds RotorGene Channel Setup FAM/Sybr, Cy5; Gain 7
72C 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.

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Thermal cycle conditions for PCR reactions on LightCycler

95C 600 sec (activation for AmpliTaq Gold polymerase)
40 cycles
95C 15 seconds
60C 30 seconds acquire fluorescent signal on F1 gain =1
72C 30 seconds
40C 30 seconds cool

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Attostar Master mixes MM150 and MM160 in RotorGene*- PCR 24 µl reactions

MM150 probe is labeled with Quasar 670-BHQ2
MM160 probe is labeled with FAM-BHQ1

*Similar cycle conditions and reaction volumes may be used on other thermal cyclers. Quasar 670 cannot be used on ABI 7700, ABI 7000, ABI 7900, ABI 7300, SmartCycler®.

RotorGene	Reagents needed for 24 ul final volume reactions:								
Reaction tube number	1	2	3	4	5	6	7	8	
Master mix MM150 or MM160	19.6	39.2	58.8	78.4	98	117.6	137.2	156.8	µl
AmpliTaq Gold polymerase	0.4	0.8	1.2	1.6	2	2.4	2.8	3.2	µl
Dispense 20 ul /tube									
Add 4 ul DNA/tube									

Attostar Master mixes MM150 or MM160 in LightCycler- PCR 24 µl reactions

LightCyler	Reagents needed for 24 µl final volume reactions:								
Reaction tube number	1	2	3	4	5	6	7	8	
Master mix T4 MM150 or MM160	19.6	39.2	58.8	78.4	98	117.6	137.2	156.8	µl
AmpliTaq Gold polymerase	0.4	0.8	1.2	1.6	2	2.4	2.8	3.2	µl
BSA 1 mg/ml	1	2	3	4	5	6	7	8	µl
Dispense 21 µl / capillary									
Add 3 µl DNA / capillary									

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Attostar primers probe detection

These primer/probe reagents are used with AttoMaster 2X Mix (Product number-AM10). AttoMaster 2X Mix c*ontains Taq polymerase (requires heat activation), dNTPs (0.4 mM) with optimal dUTP to dTTP ratio, heat labile UDG, Mg (6 mM), and buffer.*

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Thermal cycle conditions for PCR reactions on RotorGene*
*Similar cycle conditions and reaction volumes may be used on many other thermal cyclers.

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

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Thermal cycle conditions for PCR reactions on LightCycler

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

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Attostar Primers/probes PP100 and PP150 PCR 20µl reactions for RotorGene

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 PP100 or PP150	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 12 ul / reaction tube											
Add 8 ul DNA / reaction tube											

Reaction tube number	11	12	13	14	15	16	17	18	19	20	
Attostar Primer-Probe PP100 or PP150	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 12 ul / reaction tube											
Add 8 ul DNA / reaction tube											

Attostar Primers/probe PP100 PCR 20 µl reactions for LightCycler

LightCycler	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 PP100	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
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											

Reaction tube number	11	12	13	14	15	16	17	18	19	20	
Attostar Primer-Probe PP100	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
BSA 1 mg/ml	11	12	13	14	15	16	17	18	19	20	µl
Dispense 13 ul / reaction tube											
Add 7 ul DNA / reaction tube											

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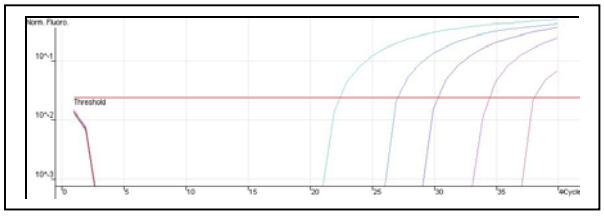
T4 multiplex reaction-Quasar 670 labeled T4 probe (PP160)

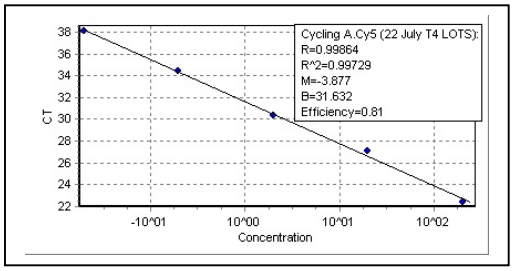
Attostar Primers/probe PP160 in RotorGene Multiplex PCR 20µl reactions

	Multiplex Reagents needed for 20 µl PCR final reaction tube volumes										
Reaction tube number	1	2	3	4	5	6	7	8	9	10	
Attostar T4 Primer-Probe PP160 Quasar 670 labeled	2	4	6	8	10	12	14	16	18	20	µl
Primer-Probe mix (10X) FAM labeled 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 µl / reaction tube											
Add 6 µl DNA / reaction tube											

Reaction tube number	11	12	13	14	15	16	17	18	19	20	
Attostar T4 Primer-Probe PP160 Quasar 670 labeled	22	24	26	28	30	32	34	36	38	40	µl
Primer-Probe mix (10X) FAM labeled 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 µl / reaction tube											
Add 6 µl DNA / reaction tube											

Typical standard curves:





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References:

1. Demerec M, Fano U. Bactereiophage-Resistant Mutants in Escherichia coli. Genetics. 1945 March; 30(2): 119–136.
2. http://www.molecular-beacons.org/Introduction.html

For technical support, contact Attostar@Attostar.com

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