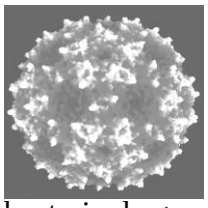
Attostar Qβ Bacteriophage
As an RNA extraction and RT-PCR control

Overview:



Bacteriophage Qβ is a small ssRNA bacteriophage isolated by Loeb (1) and described in 1961 by Loeb and Zinder. (2)

Qβ contains a 4160bp ssRNA genome. When added to a sample, Qβ bacteriophage adds a known amount of RNA. The Qβ RNA can then be extracted, amplified, and detected as a control. The Qβ bacteriophage controls for the efficiency of RNA extraction, reverse transcription, PCR amplification inhibitors, and the presence of intact amplification reagents (reverse transcriptase, Taq, buffers, dNTPs), and instrument functions (thermal cycling and fluorescence detection system).

Primers and probe are available for amplification and detection of the Qβ species. The Qβ primers and probe are specific for a 125 bp portion of the coat protein gene. The Qβ detection probe is a molecular beacon. (5)

Products	Catalog No.	Quantity

Qβ bacteriophage **BAC200** **0.5 ml**
(Store at –20C)
Typical use: add 5 ul (or dilution in water) Qβ to 100 ul samples prior to RNA extraction. Amplify and detect RT-PCR product to obtain approximate Ct=35.

Qβ bacteriophage Plasmid **PLAS205** **0.25ml**
(Store at –20C.)
Typical use: make serial 10 fold dilutions in water for standard curve, diluting 5 ul into 45 ul water.

Qβ Primers-probe FAM-BHQ1 **PP201** **0.055 ml**
(Store at –20C. 25-20 µl reactions)
Typical use: Add 2 ul to 20 ul RT-PCR reaction.
Use with one or two step RT-PCR reactions. Detection at 510nm.

Qβ Primers-probe Quasar 670-BHQ2** **PP250** **0.055 ml**
(Store at –20C. 25-20 µl reactions)
Typical use: Add 2 ul to 20 ul RT-PCR reaction.
Useful for multiplex reactions.
Use with one or two step RT-PCR reactions. Detection at 660nm.

BSA 1 mg/ml (DNase RNase free) **BSA100** **0.1ml**
(Store at –20C.)
Typical use: Add to reactions for LightCycler glass capillaries.

Use of Qβ as extraction and amplification control:

- Add 5µl Qβ bacteriophage to the sample. Proceed with RNA extraction.

- Dilutions of Qβ bacteriophage may be made to give a final RT-PCR Ct value that is about 35. At this dilution, the phage is more sensitive, i.e. more likely, for detection of a poor extraction or the presence of RT-PCR inhibitors in the reaction. If multiplex reactions are being used, this dilution is less likely to compete with the test organism RT-PCR reaction.

Use of the Qβ plasmid:

- Dilute the plasmid in water to prepare a standard curve. Common dilutions would be 10-fold from 200 to 0.02 pg/ml. The 0.02 pg/ml plasmid dilution contains 12 copies of plasmid in 2 µl. The Qβ plasmid only controls for the PCR portion of the RT-PCR reaction.

Detection of Qβ RNA:

Attostar Qβ primers and probes can be used with commercial one or two step RT-PCR master mixes. These two kits work well: Qiagen OneStep RT-PCR and Invitrogen RNA UltraSense One-Step. Each needs to be ordered from their respective manufacturer.

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Qiagen OneStep RT-PCR (Catalog number 210210):

www.qiagen.com

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Reagent volumes on RotorGene*

*Similar cycle conditions and reaction volumes may be used on many other thermal cyclers. Quasar 670 cannot be used on ABI7700 or ABI7000.

For RotorGene, no BSA added.	Reagent volumes for 20ul Qiagen OneStep RT-PCR master mixes (ul) Qiagen #210210 2.5 mM Magesium with no Mg added. With this amt Mg addition final is 5 mM Mg.										
Reaction tube number	1	2	3	4	5	6	7	8	9	10	
25 mM Magnesium	2	4	6	8	10	12	14	16	18	20	µl
RNase free water	2.4	4.8	7.2	9.6	12	14.4	16.8	19.2	21.6	24	µl
Qiagen dNTP Mix	0.8	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8.0	µl
Qiagen OneStep Enzyme Mix	0.8	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8.0	µl
Qiagen OneStep 5X buffer	4.0	8.0	12.0	16.0	20.0	24.0	28.0	32.0	36.0	40.0	µl
Attostar Primer-probe (10X)	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0	µl
	Dispense 12 µl / tube										
	RNA 8 µl / tube										

Reaction tube number	11	12	13	14	15	16	17	18	19	20	
25 mM Magnesium	22	24	26	28	30	32	34	36	38	40	µl
RNase free water	26.4	28.8	31.2	33.6	36	38.4	40.8	43.2	45.6	48	µl
Qiagen dNTP Mix	8.8	9.6	10.4	11.2	12.0	12.8	13.6	14.4	15.2	16.0	µl
Qiagen OneStep Enzyme Mix	8.8	9.6	10.4	11.2	12.0	12.8	13.6	14.4	15.2	16.0	µl
Qiagen OneStep 5X buffer	44.0	48.0	52.0	56.0	60.0	64.0	68.0	72.0	76.0	80.0	µl
Attostar Primer-probe (10X)	22.0	24.0	26.0	28.0	30.0	32.0	34.0	36.0	38.0	40.0	µl
	Dispense 12 µl / tube										
	RNA 8 µl / tube										

Cycle conditions on RotorGene

50C for 30minutes (Reverse transcriptase)
95C for 15 minutes (Activation of Taq and inactivation of Reverse transcriptase)
45 cycles
95C 15 seconds
55C 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.

Reagent volumes for LightCycler

LightCycler with BSA added	Reagent volumes for 20ul Qiagen OneStep RT-PCR master mixes (ul) Qiagen #210210 2.5 mM Magesium with no Mg added. With this amt Mg addition final is 5 mM Mg.										
Reaction tube number	1	2	3	4	5	6	7	8	9	10	
25 mM Magnesium	2	4	6	8	10	12	14	16	18	20	µl
RNase free water	1.4	2.8	4.2	5.6	7	8.4	9.8	11.2	12.6	14	µl
Qiagen dNTP Mix	0.8	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8.0	µl
Qiagen OneStep Enzyme Mix	0.8	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8.0	µl
Qiagen OneStep 5X buffer	4.0	8.0	12.0	16.0	20.0	24.0	28.0	32.0	36.0	40.0	µl
BSA 1 mg/ml	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	µl
Attostar Primer-probe PP201 (10X)	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0	µl
	Dispense 12 ul / reaction tube										
	RNA 8 µl / tube										

Reaction tube number	11	12	13	14	15	16	17	18	19	20	
25 mM Magnesium	22	24	26	28	30	32	34	36	38	40	µl
RNase free water	15.4	16.8	18.2	19.6	21	22.4	23.8	25.2	26.6	28	µl
Qiagen dNTP Mix	8.8	9.6	10.4	11.2	12.0	12.8	13.6	14.4	15.2	16.0	µl
Qiagen OneStep Enzyme Mix	8.8	9.6	10.4	11.2	12.0	12.8	13.6	14.4	15.2	16.0	µl
Qiagen OneStep 5X buffer	44.0	48.0	52.0	56.0	60.0	64.0	68.0	72.0	76.0	80.0	µl
BSA 1 mg/ml	11.0	12.0	13.0	14.0	15.0	16.0	17.0	18.0	19.0	20.0	µl
Attostar Primer-probe PP201 (10X)	12.0	14.0	16.0	18.0	20.0	22.0	24.0	26.0	28.0	30.0	µl
	Dispense 12 ul / reaction tube										
	RNA 8 µl / tube										

Cycler conditions for LightCycler

50C for 30 minutes (Reverse transcriptase)
95C for 15 minutes (Activation of Taq and inactivation of Reverse transcriptase)
45 cycles
    95C 15 seconds
    55C 30 seconds acquire fluorescent signal on F1 gain =1
    72C 30 seconds
40C 30 seconds cool

Invitrogen RNA UltraSense One-Step RT-PCR (Catalog number 11732-927)

www.invitrogen.com

Reagent volumes on RotorGene

RotorGene	Reagent volumes for 20 ul Invitrogen RNA UltraSense One-Step Quantitatve RT-PCR Cat no 11732-927.										
Reaction tube number	1	2	3	4	5	6	7	8	9	10	
RNA UltraSense Enzyme	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	µl
RNA UltraSense 5X buffer	4.0	8.0	12.0	16.0	20.0	24.0	28.0	32.0	36.0	40.0	µl
Attostar Primer-probe PP201	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0	µl
	Dispense 7 ul/tube										
	Dispense 13 ul RNA/tube										
Reaction tube number	11	12	13	14	15	16	17	18	19	20	
RNA UltraSense Enzyme	11.0	12.0	13.0	14.0	15.0	16.0	17.0	18.0	19.0	20.0	µl
RNA UltraSense 5X buffer	44.0	48.0	52.0	56.0	60.0	64.0	68.0	72.0	76.0	80.0	µl
Attostar Primer-probe PP201	22.0	24.0	26.0	28.0	30.0	32.0	34.0	36.0	38.0	40.0	µl
	Dispense 7 ul/tube										
	Dispense 13 ul RNA/tube										

Cycle conditions on RotorGene

50C for 30minutes (Reverse transcriptase)
95C for 15 minutes (Activation of Taq and inactivation of Reverse transcriptase)
45 cycles
- 95C 15 seconds
- 55C 30 seconds RotorGene Channel Setup FAM/Sybr, Cy5; Gain 7
- 72C 30 seconds

Reagent volumes on LightCycler

LightCycler with BSA added	Reagent volumes for 20 ul Invitrogen RNA UltraSense One-Step Quantitatve RT-PCR Cat no 11732-927.										
Reaction tube number	1	2	3	4	5	6	7	8	9	10	
RNA UltraSense Enzyme	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	µl
RNA UltraSense 5X buffer	4.0	8.0	12.0	16.0	20.0	24.0	28.0	32.0	36.0	40.0	µl
BSA 1 mg/ml	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	µl
Attostar Primer-probe PP201	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0	µl
	Dispense 8 ul/tube										
	Dispense 12 ul RNA/tube										
Reaction tube number	11	12	13	14	15	16	17	18	19	20	
RNA UltraSense Enzyme	11.0	12.0	13.0	14.0	15.0	16.0	17.0	18.0	19.0	20.0	µl
RNA UltraSense 5X buffer	44.0	48.0	52.0	56.0	60.0	64.0	68.0	72.0	76.0	80.0	µl
BSA 1 mg/ml	11.0	12.0	13.0	14.0	15.0	16.0	17.0	18.0	19.0	20.0	µl
Attostar Primer-probe PP201	22.0	24.0	26.0	28.0	30.0	32.0	34.0	36.0	38.0	40.0	µl
	Dispense 8 ul/tube										
	Dispense 12 ul RNA/tube										

Cycle conditions on LightCycler:

50C for 30 minutes (Reverse transcriptase)
95C for 15 minutes (Activation of Taq and inactivation of Reverse transcriptase)
45 cycles
    95C 15 seconds
    55C 30 seconds acquire fluorescent signal on F1 gain =1
    72C 30 seconds
40C 30 seconds cool

Multiplex reactions to detect Qβ RNA:

The Qβ RNA and test organism RNA may be detected using a multiplex reaction using Quasar 670 labeled probe (PP250) and FAM labeled test organism probe.

Reagent volumes multiplex Qiagen OneStep RT-PCR

For RotorGene, no BSA added.	Reagent volumes for 20ul Qiagen OneStep RT-PCR master mixes (ul) Qiagen #210210 2.5 mM Magesium with no Mg added. With this amt Mg addition final is 5 mM Mg.										
Reaction tube number	1	2	3	4	5	6	7	8	9	10	
25 mM Magnesium	2	4	6	8	10	12	14	16	18	20	µl
RNase free water	0.4	0.8	1.2	1.6	2	2.4	2.8	3.2	3.6	4	µl
Qiagen dNTP Mix	0.8	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8.0	µl
Qiagen OneStep Enzyme Mix	0.8	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8.0	µl
Qiagen OneStep 5X buffer	4.0	8.0	12.0	16.0	20.0	24.0	28.0	32.0	36.0	40.0	µl
Attostar Primer-probe FAM label (10X)	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0	µl
Attostar Primer-probe PP250 (10X)	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0	µl
	Dispense 12 µl / tube										
	RNA 8 µl / tube										
Reaction tube number	11	12	13	14	15	16	17	18	19	20	
25 mM Magnesium	22	24	26	28	30	32	34	36	38	40	µl
RNase free water	4.4	4.8	5.2	5.6	6	6.4	6.8	7.2	7.6	8	µl
Qiagen dNTP Mix	8.8	9.6	10.4	11.2	12.0	12.8	13.6	14.4	15.2	16.0	µl
Qiagen OneStep Enzyme Mix	8.8	9.6	10.4	11.2	12.0	12.8	13.6	14.4	15.2	16.0	µl
Qiagen OneStep 5X buffer	44.0	48.0	52.0	56.0	60.0	64.0	68.0	72.0	76.0	80.0	µl
Attostar Primer-probe FAM label (10X)	22.0	24.0	26.0	28.0	30.0	32.0	34.0	36.0	38.0	40.0	µl
Attostar Primer-probe PP250 (10X)	22.0	24.0	26.0	28.0	30.0	32.0	34.0	36.0	38.0	40.0	µl
	Dispense 12 µl / tube										
	RNA 8 µl / tube										

Reagent volumes multiplex Invitrogen RNA UltraSense One-Step RT-PCR

RotorGene	Reagent volumes for 20 ul Invitrogen RNA UltraSense One-Step Quantitatve RT-PCR Cat no 11732-927.										
Reaction tube number	1	2	3	4	5	6	7	8	9	10	
RNA UltraSense Enzyme	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	µl
RNA UltraSense 5X buffer	4.0	8.0	12.0	16.0	20.0	24.0	28.0	32.0	36.0	40.0	µl
Attostar Primer-probe FAM labeled (10X)	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0	µl
Attostar Qbeta Primer-probe Quasar 670 labeled PP250	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0	µl
	Dispense 9 ul/tube										
	Dispense 11 ul RNA/tube										
Reaction tube number	11	12	13	14	15	16	17	18	19	20	
RNA UltraSense Enzyme	11.0	12.0	13.0	14.0	15.0	16.0	17.0	18.0	19.0	20.0	µl
RNA UltraSense 5X buffer	44.0	48.0	52.0	56.0	60.0	64.0	68.0	72.0	76.0	80.0	µl
Attostar Primer-probe FAM labeled (10X)	22.0	24.0	26.0	28.0	30.0	32.0	34.0	36.0	38.0	40.0	µl
Attostar Qbeta Primer-probe Quasar 670 labeled PP250	22.0	24.0	26.0	28.0	30.0	32.0	34.0	36.0	38.0	40.0	µl
	Dispense 9 ul/tube										
	Dispense 11 ul RNA/tube										

References:

1. Loeb T. Isolation of a Bacteriophage Specific for the F+ and Hfr Mating Types of Escherichia coli K-12. Science (1960) 131: 932-933.
2. Loeb T and Zinder ND. A Bacteriophage Containing RNA. Proc. N.A.S. (1961) 47: 282-289.
3. http://www.molecular-beacons.org/Introduction.html

For technical support, contact Attostar@Attostar.com

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