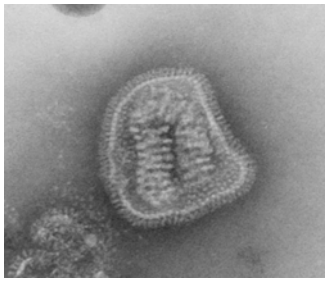
Influenza B viruses
Detection with real time RT-PCR reagents

Overview:



Ref. 2

Influenza A occurs in epidemics every year. The virus contains the potential for lethal effect and must always be taken seriously. Influenza has an abrupt onset with fever with chills, body aches, and sore throat, followed quickly by a cough that may be painful. Rhinitis or discomfort with painful stuffy nose. Headache, malaise, myalgia are common. The illness in adults may persist with fevers for 5 to 7 days, followed by several weeks of convalescence including a persistent cough. Progression to pneumonia is a common complication that may be severe, primarily in adults.

Influenza B causes similar annual epidemics, but usually milder disease.

Primers and probe are available for amplification and detection of Influenza A and B viruses. The Influenza A virus primers are specific for an 84 bp portion of the matrix gene. The conserved probe sequence detects most, but not all, Influenza A. The Influenza B virus primers amplify a 148 bp segment of the matrix protein. The detection probes for Influenza viruses A and B are molecular beacons (1)

Products

Products	Catalog No.	Quantity
Influenza B matix FAM-BHQ1	**PP600**	**0.055ml**

Primers and probe.
Store at –20C. 25-20 µl reactions
Typical use: Add 2 µl to 20 µl RT-PCR reaction. Use with one or two step RT-PCR reactions. Detect at 510nm.

Influenza B Plasmid 200 pg/ml	**PLAS600**	**0.25ml**

Store at –20C.
Typical use: make serial 10 fold dilutions in water for standard curve, diluting 5 µl into 45µl water.

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Detection Influenza A and B RNA

Attostar Influenza virus 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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Use of the Influenza A and B plasmids:

Dilute the plasmid in water 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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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.

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 PP500 or PP600 (	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 PP500 or PP600 (	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 condtions on RotorGene

50C for 30minutes (Reverse transcriptase)
95C for 15 minutes (Activation of Taq and inactivation of Reveres 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)

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 PP500 or PP600	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 PP500 or PP600	22.0	24.0	26.0	28.0	30.0	32.0	34.0	36.0	38.0	40.0	µl
	Dispense 12 ul / reaction tube										
	RNA 8 µl / tube										

Cycler conditions for LightCycler

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 acquire fluorescent signal on F1 gain =5
    72C 30 seconds
40C 30 seconds cool

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

www.invitrogen.com

Reagent volumes for 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 PP500 or PP600	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 PP500 or PP600	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 for 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.

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Reagent volumes for 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 PP500 or PP600	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 PP500 or PP600	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 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 =5
- 72C 30 seconds

40C 30 seconds cool

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Extraction / amplification control with Qβ bacteriophage

Adding Qβ bacteriophage (BAC200) to the sample provides RNA for extraction, amplification, and reaction condition RT and PCR controls.

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, intact amplification reagents (reverse transcriptase, DNA polymerase, buffers, dNTPs), and instrument function (thermal cycling and fluorescent detection system).

The Qβ RNA may be detected in a separate RT-PCR reaction using FAM labeled probe (PP201). Or 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.

Please refer to the Qβ BAC200, PP201, and PP250 product literature.

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

- Add 5µl Qβ bacteriophage to the sample. Proceed with RNA extraction. Dilutions of the 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, to detect a poor extraction or the presence of RT-PCR inhibitors in the reaction.

  Please refer to the Qβ BAC200, PP201, and PP250 product literature.

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Reference:

1) http://www.molecular-beacons.org/Introduction.html
2) Image from http://phil.cdc.gov/Phil/details.asp Dr. Erskine. L. Palmer; Dr. M. L. Martin

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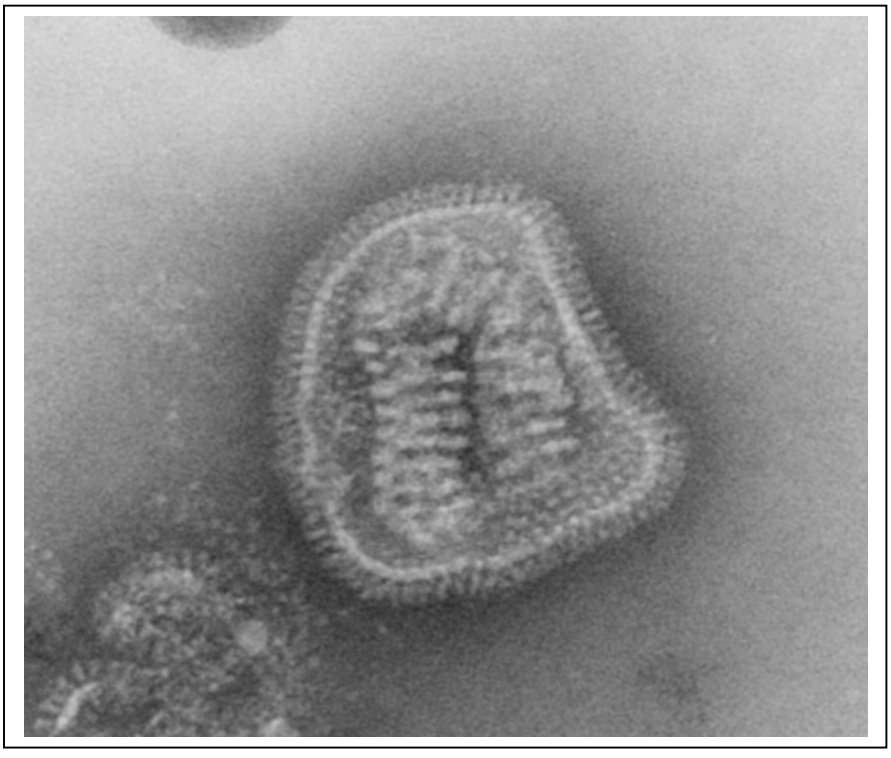
For technical support, contact Attostar@Attostar.com

Enlarged image:

Influenza electron micrograph. (2)



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