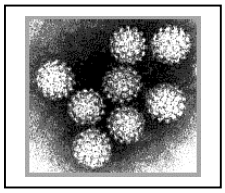
Polyomaviruses BK and JC
PCR reagents

Overview:



Polyomaviruses are ubiquitous dsDNA human and animal viruses. Most adults have been infected and harbor latent virus with no serious consequences. However immune suppression may result in serious diseases including JC associated progressive multifocal leukoencephalopathy (PML) and BK associated graft failure in renal transplant patients and systemic, lung, liver, and brain disease in other immune-compromised persons. Wu and Ki are newly described human respiratory polyoma viruses.

Polyomaviruses may infect many species including: monkeys, mouse, cows, rabbits, birds, and others. Infections of species other than the natural host may cause tumors, i.e. SV40, a monkey virus, may cause sarcomas and leukemia in hamsters.

Dr Sara Stewart 1906-1976 (Georgetown University's first medical doctor) and Dr Bernice Eddy 1903-1989 were the first to describe polyomaviruses (1). They showed the viruses to cause tumors in many animals.

Master mix, primers, probes, and buffer are available for amplification and detection of the BK and JC. The BK primers are specific for a 140 bp portion of coat protein VP2. This amplification reagent detects BK not JC. The BK detection probe is a molecular beacon. The JC primers are specific for a 140 bp portion of coat protein VP2. The JC probe detects JC not BK. The BK and JC detection probes are molecular beacons. (2)

Products

Products	Catalog No.	Quantity	Price
BK Master mix FAM-BHQ1	**MM5700**	**0.175ml**	**$32**
Store at –20C. 8 reactions *Typical use: Add Taq and DNA, then thermal cycle.* *Master mix contains buffer, dNTPs, primers, and probe.* *Detection at 510nm.*			
BK FAM-BHQ1 Primer-probe	**PP5700**	**0.055ml**	**$80**
Store at –20C. 20 reactions *Typical use: Add 2X master mix and DNA, then thermal cycle.* *Attostar reagent contains primers and probe.* *Detection at 510nm.*			
JC Master mix FAM-BHQ1	**MM5800**	**0.175ml**	**$32**
Store at –20C. 8 reactions *Typical use: Add Taq and DNA, then thermal cycle.* *Master mix contains buffer, dNTPs, primers, and probe.* *Detection at 510nm.*			
JC FAM-BHQ1 Primer-probe	**PP5800**	**0.055ml**	**$80**
Store at –20C. 20 reactions *Typical use: Add 2X master mix and DNA, then thermal cycle.* *Attostar reagent contains primers and probe.* *Detection at 510nm.*			
BK Plasmid	**PLAS5700**	**0.25ml**	**$35**
Store at –20C. *Typical use: make serial 10 fold dilutions in TE for standard curve, diluting 5 ul into 45 ul water.*			
JC Plasmid	**PLAS5800**	**0.25ml**	**$35**
Store at –20C. *Typical use: make serial 10 fold dilutions in TE for standard curve, diluting 5 ul into 45 ul water.*			

Detection of Polyomavirus DNA:

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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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Use of the Polyomavirus plasmids:

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 20 copies of plasmid in 2 μl

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Attostar Master mix MM5700 or MM5800 in RotorGene*- PCR 24 µl reactions

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

Reagents needed for 24 µl final volume reactions:									
Reaction tube number	1	2	3	4	5	6	7	8	
Master mix MM5700 or MM5800	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 µl /tube									
Add 4 µl DNA/tube									

Attostar Master mix MM5700 or MM5800 in LightCycler- PCR 24 µl reactions

	Reagents needed for 24 µl final volume reactions:								
	1	2	3	4	5	6	7	8	
Master mix MM5700 or MM5800	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 PP5700 or PP5800 in RotorGene-PCR 20µl reactions

	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 PP5700 or PP5800	2	4	6	8	10	12	14	16	18	20	µl
2 x master mix	12	24	36	48	60	72	84	96	108	120	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 PP5700 or PP5800	22	24	26	28	30	32	34	36	38	40	µl
2 x master mix	132	144	156	168	180	192	204	216	228	240	µl
Dispense 14 ul / reaction tube											
Add 6 ul DNA / reaction tube											

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Attostar Primers/probe PP5700 or PP5800 in LightCycler-PCR 20 µl reactions

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	LightCycler Reagents needed for 20 µl PCR final reaction tube volumes										
Reaction tube number	1	2	3	4	5	6	7	8	9	10	
Attostar Primer-Probe PP5700 or PP5800	2	4	6	8	10	12	14	16	18	20	µl
2 x master mix	12	24	36	48	60	72	84	96	108	120	µl
BSA 1 mg/ml	1	2	3	4	5	6	7	8	9	10	µl
Dispense 15 µl / reaction tube											
Add 5 µl DNA / reaction tube											

Reaction tube number	11	12	13	14	15	16	17	18	19	20	
Attostar Primer-Probe PP5700 or PP5800	22	24	26	28	30	32	34	36	38	40	µl
2 x master mix	132	144	156	168	180	192	204	216	228	240	µl
BSA 1 mg/ml	11	12	13	14	15	16	17	18	19	20	µl
Dispense 15 µl / reaction tube											
Add 5 µl DNA / reaction tube											

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Polyomavirus and T4 multiplex reaction (PP5700 or PP5800 and PP160)

RotorGene Multiplex PCR 20µl reactions

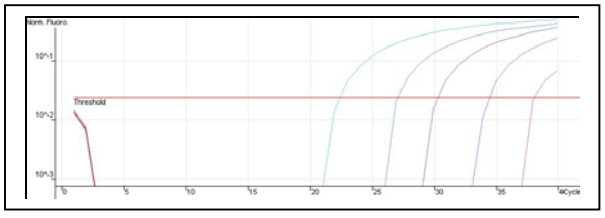
Brief procedure for use of T4 as extraction and amplification control:

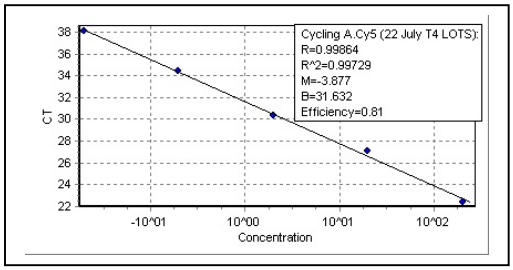
- Add 5µl bacteriophage to the sample. Proceed with DNA extraction. Dilutions of the bacteriophage may be made to give a final PCR Ct value that is about 30-35. At this dilution, the phage is more sensitive, i.e. more likely, to detect a poor extraction or the presence of PCR inhibitors in the reaction. Please refer to the PP160 product literature.

	Multiplex Reagents needed for 20 µl PCR final reaction tube volumes										
Reaction tube number	1	2	3	4	5	6	7	8	9	10	
T4 Primer Probe PP160	2	4	6	8	10	12	14	16	18	20	µl
Polyoma Primer-Probe PP5700 or PP5800	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	
T4 Primer Probe PP160	22	24	26	28	30	32	34	36	38	40	µl
Polyomavirus Primer-Probe PP5700 or PP5800	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 curve figures:





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Reference:

1) Eddy, BE and Stewart SE, Characteristics of the SE Polyoma Virus, Am J Public Health Nations Health 1959 November, 49: 1491-1492.
2) http://www.molecular-beacons.org/Introduction.html

For technical support, contact Attostar@Attostar.com