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qPCR Method for Quantification of Human Polyomaviruses

Microbial Source Tracking

Human Polyomavirus (HPyVs) Taqman PCR

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Reagents

Reagent	Manufacturer	Cat. No.
TaqMan Universal PCR Master Mix, No AmpErase UNG	Applied Biosystems	4364343
MGBNFQ probe	Applied Biosystems	4316033

Oligonucleotide		Sequence	Length (bp)
Forward	SM2	5'-AGT CTT TAG GGT CTT CTA CCT TT-3'	23
Reverse	P6	5'-GGT GCC AAC CTA TGG AAC AG-3'	20
Probe	KGJ3	5'-(FAM)-TCA TCA CTG GCA AAC AT-(MGBNFQ)-3'	17

Standard curve

Plasmids containing either the BK virus (ATCC VR-837) or JC virus (ATCC VR-1583) insert are used for the standard curve. Insert copy numbers are estimated by multiplying the average DNA concentration by Avogadro's number then dividing by the product of the insert length and average weight of a base pair (1). To produce a standard curve, the recombinant plasmid DNA is serially diluted in nuclease-free reagent grade water to a final concentration ranging from 10^2 to 10^6 gene copies•μl^{-1}.

qPCR reaction master mix

Reagent	Volume (µl)	Final concentrations
Taqman Master Mix	25.0	n/a
SM2 (10µM)	2.5	0.5 µM
P6 (10µM)	2.5	0.5 µM
KGJ3 (10µM)	2.0	0.4 µM
Sterile water	13.0	n/a
Template	5.0	n/a
Total	50.0	n/a

qPCR reaction conditions

Amplification was performed in the 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA).

	Temperature (°C)	Time	
DNA polymerase activation	95	10 min	
Denaturing	95	15 sec	45 cycles
Anneal	55	20 sec	
Extension	60	60 sec	

In general, Applied Biosystems default settings for the threshold cycle (Ct) are used for data analysis. However, amplification curves should be examined to ensure the automatic Ct is appropriate for the samples processed.

Questions or concerns

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REFERENCES

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