Validation of a Highly Sensitive qPCR Assay for the Detection of Plasma Cell-free Epstein-Barr virus DNA in Nasopharyngeal Carcinoma Diagnosis

Final protocol for quantitative detection of plasma cell-free EBV-DNA using our qPCR assay

We propose the optimized protocol for our qPCR assay as detailed below.

Components	Working concentration	Final concentration	Unit	Reaction Volume (μL)
Quantitec Probe PCR Master Mix	2	1	X	10
Forward Primer	10	0.2	μM	0.4
Reverse Primer	10	0.2	μM	0.4
Probe	10	0.05	μM	0.1
DMSO	100	2.5	%	0.5
DNA Template				8.6
Total Volume				20

Supplementary Table 1. Optimal concentrations of qPCR components.

Step		Temperature (°C)	Time	Time Unit
Initial denaturation		95	15	Min
45 cycles	Denaturation	94	15	sec
	Annealing	63	30	sec
	Extension	72	30	sec
Holding stage		25	∞	

Supplementary Table 2. Optimal thermocycling program for plasma cell-free EBV-DNA qPCR assay.