

Supplementary Table S1: Characteristics of CHIKV-specific primers and probe in this study

	T _m (°C)	GC content (%)	ΔG of self-dimer (kcal/mol)	ΔG of hetero-dimer (kcal/mol)	ΔG of hairpin (kcal/mol)
F	54.7	52.5	-3.61	-6.90 (R); -4.95 (P)	1.34
R	58.6	60.0	-5.12	-6.69 (F); -4.12 (P)	1.24
P	48.6	47.1	-6.30	-4.95 (F); -4.12 (R)	0.43

F=forward primer; R=reverse primer; P=probe; T_m=melting temperature; ΔG=Gibbs free energy change. For hetero-dimer, the primers or probe in bracket indicate the formation of hetero-dimers between 25 them. Only the highest negative values of ΔG for each dimers and hairpins were recorded.

Supplementary Table S2: Characteristics of recombinant plasmid designed in this study

Vector	Nucleotide sequence (5' → 3')	Size of recombinant plasmid (bp)	Length of target sequence (bp)
pUC57-mini	CCAATGATGCGGCCGC <u>TAATACGACTCACTATAGGG</u> AGAAGCCAG GATGCTGAAAAAGAA <u>GCAGAGGAAGAACGAGAAAGCAAACTGACTCATG</u> <u>AGGCTCTACCA</u> CCCTACAGGCAGCACAGGAAGATGTCAGGTCGAAAT <u>CGACGTGGAACA</u> ACTTGAGGATAGAGCTGGTCTGGAATAATAGAGACT <u>CCGAGAGGCGCTATTAAAGTTACTGCCCACTAACAGACCAC</u> GTCTGTGG GGGAGTACCTGGTACTTTCCCGCAGACCGTACTACGTAGCCAAAAGCTT AGCCTGATTACGCTTTGGCGGAGCAAGTGAAGACGTGCACGCACAGCG GACGAGCAGGGAGGTATGCGGTGCAAGCGTACGACGGCAGAGTCCTAGT GCCCTCAGGCTACGCAATCTCGCTGAAGACTTCCAGAGCCTATCGTGTT AACGTGCTTCCGCGGCTGGGTGAAGCAACTGCAAAATCGACTATCGTGGA CACGAGGTGATGACCGCAGCTGCTTCCAGGGTCTGACAAGGAAAGGCG TGTAACGCGTGAGACAGAAAGTGAACGAAACCCACTGTGGCAGCTGCA TTTGCTGAGAGCAATACAGGTGGTGACATTGTGCACTTGGCCATGGTGG CTGCTTTTAACATCCAGCCAGGTTTACTGATTGGATTCTACTGAGGAGG AAGTGGAGCAATCAGGAAAGCAGATTGCTTGGCGTTGCGTTAGCACTTA TAACAGTGGCAATGAGAGATTTGAACATGAGTATACCAACATTACTCAAC TCCGAGCCATGGCCTGGCTCTTGCTGAGAGCCGTGTTGAAGGGACGG TTAGCTCCTTGTCTGCCACTTGTAGCTTGTGCTCCAGGGCTCAGA ATAGTGGGATAGATGTGGTGAGAATAGGTGTGCTAACCTGGGAATCC TCTCACTATTGAAAGAGAGGAGCAATGCAATGGCAGAATTCC <u>GGATCC</u> AT CGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAA AAAGGCCGCTTGTGCGGTTTTCCATAGGCTCCGCCCCCTGACGAG CATCAAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGAC TATAAAGATACAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCC TGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTCGG GAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCCGT GTAGGTCTGCTCCTCAAGCTGGGCTGTGTGACGAAACCCCGTTCAG CCGACCGCTGCGCCTTATCCGTAATATCGTCTTGAGTCCAACCCGG TAAGACAGGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAG CAGAGCGAGGTATGTAGGCGGTGTACAGAGTCTTGAAGTGGTGGCCT AACTACGGCTACACTAGAAGAACAGTATTGGTATCTGCGCTCTGCTGAA GCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAA CCACCGCTGGTAGCGGTGGTTTTTTGTTGCAAGCAGAGATTACGCGC AGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGA CGCTCAGTGAACGAAACTCACGTAAAGGATTTTGGTCTAGAGATTAT CAAAAAGGATCTTACCTAGATCCTTTAAATTAAAAATGAAGTTTAAAT	2810	174
	CAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTA ATCAGTAGGACCTATCTCAGCGATCTGTCTATTTCGTTTCATCCATAGTT GCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGC CCAGTGCTGCAATGATACCGCGAGACCCACGCTACCGGCTCCAGATTTATCA GCAATAAACCCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTT ATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGAAGCTAGAGTAAGTAGTTC GCCAGTTAATAGTTTGCAGAACGTTGTTGCCATTGCTACAGGCATCGTGGTGT ACGCTCGCTGTTGGTATGGCTTCAATCAGCTCCGTTCCCAACGATCAAGGCG AGTTACATGATCCCCATGTTGTGCAAAAAAGCGTTAGCTCCTTCGGTCTCC GATCGTTGTGCAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGC ACTGCATAATTCTTACTGTATGCCATCCGTAAGATGCTTTTCTGTGACTGGT GAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCT TGCCCGGCTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAGT GCTCATATTGGAAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCT GTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCAACTGATCTTCAGCATC TTTTACTTTACACGCGTTTCTGGGTGAGCAAAAAACAGGAAGGCAAAATGCCG CAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTT TTCAATATTATTGAAGCATTTATCAGGGTATTGTCTCATGAGCGGATACATA TTTGAATGTATTTAGAAAAATAACAAATAGGGGTTCGCGCGACATTTCCCCGA AAAGTGCCACCTGACGTC		

The nucleotide sequence of recombinant plasmid was shaded with grey colour and underlined with different lines. Single underline indicates T7 promoter, dotted underline indicates target sequence and wave underline indicates restriction site for BamH1.

Supplementary Table S3: Comparison of Ct values between developed real-time qRT-PCR assay and commercial GenoAmp Triplex real-time RT-PCR Zika/Den/Chiku assay

No. of sample	qRT-PCR assay	trioplex assay
	Ct value	Ct value
1	26.50	23.89
2	Undetected	Undetected
3	36.25	37.54
4	26.15	24.54
5	22.75	19.90
6	25.76	23.88
7	Undetected	Undetected
8	36.83	40.00
9	Undetected	Undetected

Kappa measure of agreement ($\kappa=1.000$, $p=0.003$).