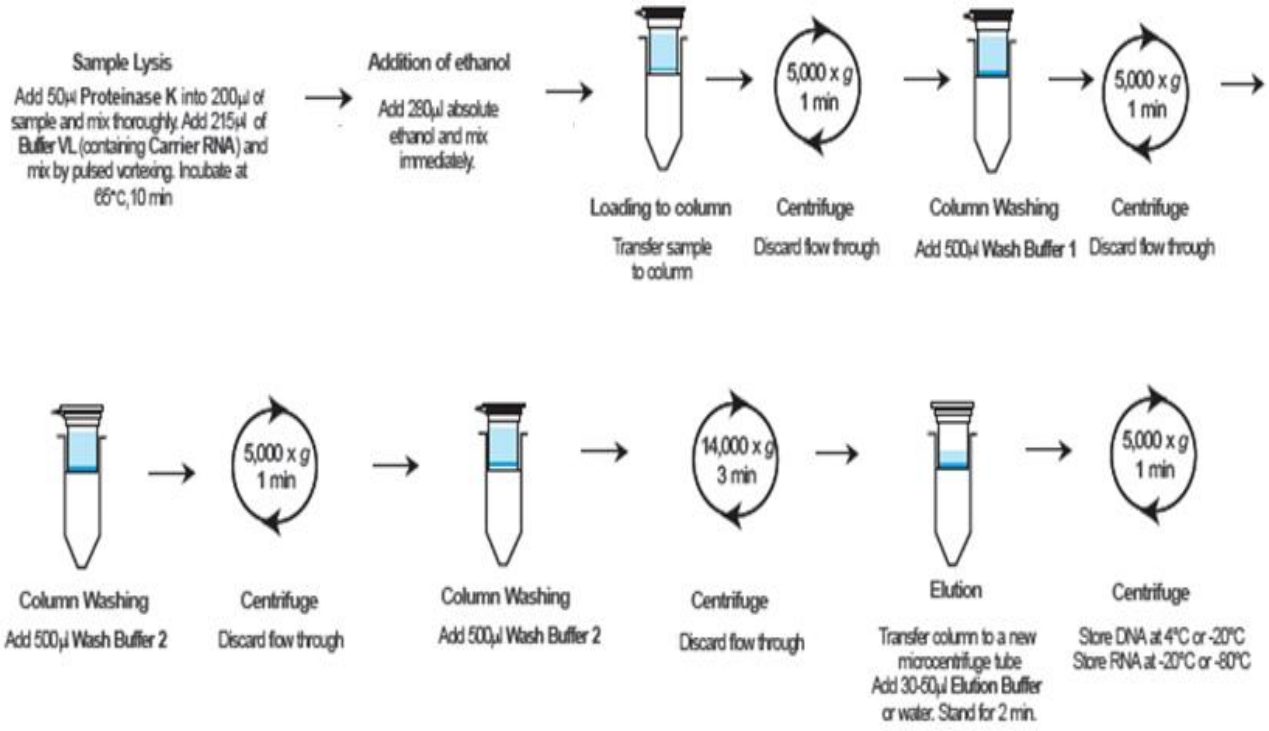
Comparison Test of Performance of Proteinase K & Carrier RNA Stored Cold and Stored Room Temp.
Viral RNA Extraction Test

Objective
To compare the performance of cold-stored Proteinase K & Carrier RNA (-20ºC) and room temperature-stored Proteinase K & Carrier RNA (25-28ºC) used in viral RNA extraction test.

Passing Criteria
The amplification of extracted RNA using conventional PCR showed **positive results with 500bp band**.
The amplification of extracted RNA using real-time PCR showed positive results with the **difference of Ct value between two Proteinase Ks and two Carrier RNAs is less than 3**.

Samples
- Dengue virus spiked into human plasma

Protocol
5µl of dengue virus with concentration ±100ng/µl is spiked into 195µl 2X diluted plasma.



Vivantis Technologies Sdn Bhd 587389-D
Revongen Corporation Center
No12A, Jalan TP5, Taman Perindustrian UEP,
47600 Subang Jaya, Selangor Darul Ehsan, Malaysia.
E : info@vivantechnologies.com
W: www.vivantechnologies.com
T: +6 03 8025 1603
F: +6 03 8025 1637/1354

Results

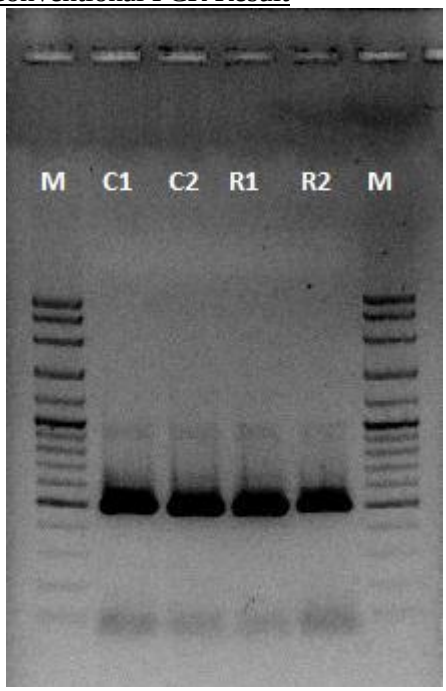
Downstream Application

The extracted RNA was performed with 2-step conventional PCR:

- Reverse transcription test to transcript the RNA into cDNA
- Conventional PCR to amplify the cDNA.

The extracted RNA was also performed with one-step real-time PCR. Both tests were performed using dengue viral primer.

Conventional PCR Result



Legend:
M: 100bp plus DNA ladder

C1&C2: Amplification product using extracted RNA which used cold-stored Proteinase K and Carrier RNA in extraction

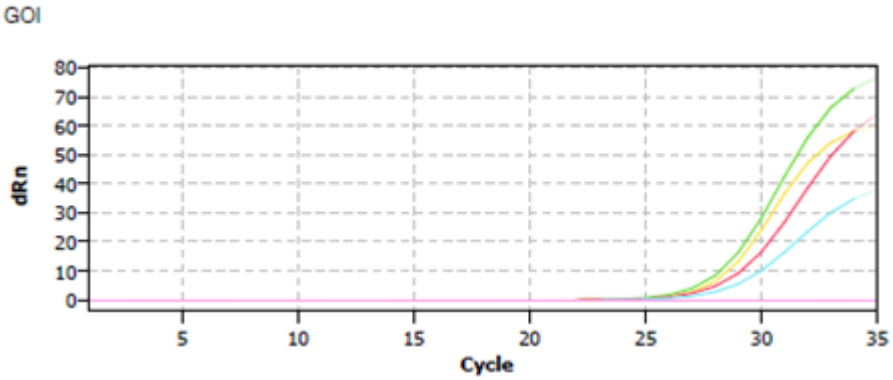
R1&R2: Amplification product using extracted RNA which used room temperature-stored Proteinase K and Carrier RNA in extraction

Figure 1: 2µl of extracted DNA was used for reverse transcription and 5µl of cDNA is used in amplification. 5µl of PCR product was loaded into 1% TBE gel. The expected band size is 500bp.

Vivantis Technologies Sdn Bhd 587389-D
Revongen Corporation Center
No12A, Jalan TP5, Taman Perindustrian UEP,
47600 Subang Jaya, Selangor Darul Ehsan, Malaysia.
E : info@vivantechnologies.com
W: www.vivantechnologies.com
T: +6 03 8025 1603
F: +6 03 8025 1637/1354

vivantis

Real-time PCR Result



Well		Sample n	Sample t	Gene	Ct	Mean Ct	Conc. Std	Mean Co	Std.Dev.	Std.Dev.
G6		RT Deng	Unknown		27.69	27.69			0	
G5		RT Deng	Unknown		27.03	27.03			0	
G4		Cool Den	Unknown		26.25	26.25			0	
G3		Cool Den	Unknown		26.77	26.77			0	
G8		-ve	Unknown		No Ct					

Mean Ct value for RT Dengue	27.360
Mean Ct value for Cool Dengue	26.510
Difference Ct value between RT and Cool	**0.850**

Figure 2: 2µl of extracted RNA was used for real-time amplification. According to the graph and table on top, the difference in Ct value between two different Proteinase Ks and Carrier RNAs is 0.850.

Conclusion

Dengue viral samples were extracted using GF-1 Viral Nucleic Acid Extraction kit. There was no significant difference showed in the performance of Proteinase K & Carrier RNA that was stored in either cold or room temperature condition as the results of amplifications of extracted RNA using conventional PCR showed no significant different for bands; and using real-time PCR showed that the differences between the two Proteinase Ks and two Carrier RNAs are within 1Ct value. The sensitivity of the conventional and real-time assay was not affected by the use of room temperature-stored Proteinase K and Carrier RNA.

Prepared by,
Vivantis Technical Team
29th June 2016

Vivantis Technologies Sdn Bhd 587389-D
Revongen Corporation Center
No12A, Jalan TP5, Taman Perindustrian UEP,
47600 Subang Jaya, Selangor Darul Ehsan, Malaysia.
E : info@vivantechnologies.com
W: www.vivantechnologies.com
T: +6 03 8025 1603
F: +6 03 8025 1637/1354