VariSafe™ RNA
Application Note

Varizymes
OPTIONS AMPLIFIED

VariSafe RNA products contain single-stranded RNA encapsulated in phage coat protein that is resistant to nucleases and can serve as process or internal controls for a variety of applications.

Development of VariSafe RNA controls: Briefly, this method involves purified MS2 phage-like particles engineered to package single-stranded RNA (up to 1.5 kb) and protect it from ubiquitous nucleases present in sample matrices. For example, to generate the SARS-CoV-2 VariSafe RNA control (Catalog #1002), a 1000 bp region of the N gene and a 140 bp region of the human RNase P gene RPP30 were cloned, transcribed, and packaged in MS2 phage like particles (Figure 1). These can then be used to simulate a positive control to aid in assay development and optimization.

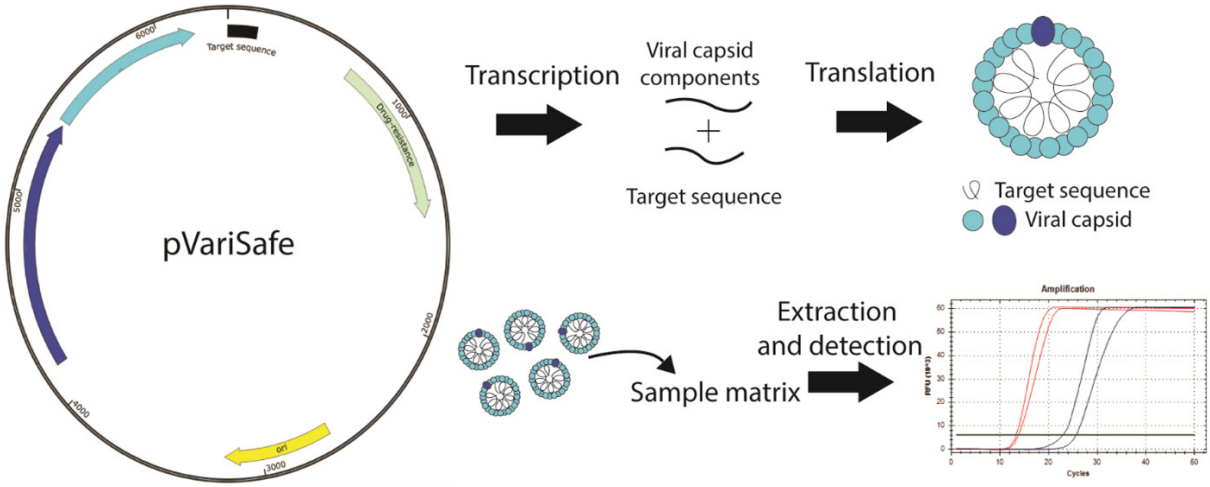

Figure 1: Overview of VariSafe RNA technology

Protection of VariSafe RNA from nuclease degradation: Encapsulated VariSafe RNA are protected from degradation by nucleases. 2.2E8 copies of a custom VariSafe RNA were incubated at 37 °C with and without RNase I and with ("Lysed") and without ("Encapsulated") a pre-treatment of 85 °C for 5 minutes to lyse the phage-like particles and then used as template in a real-time TaqMan RT-PCR assay. The results presented in Figure 2 show that lysis of the particles facilitates amplification (without RNase, encapsulated samples amplify slower than lysed samples) and that VariSafe RNAs are protected from degradation by RNase I (lysed samples amplify much slower if RNase I is present).

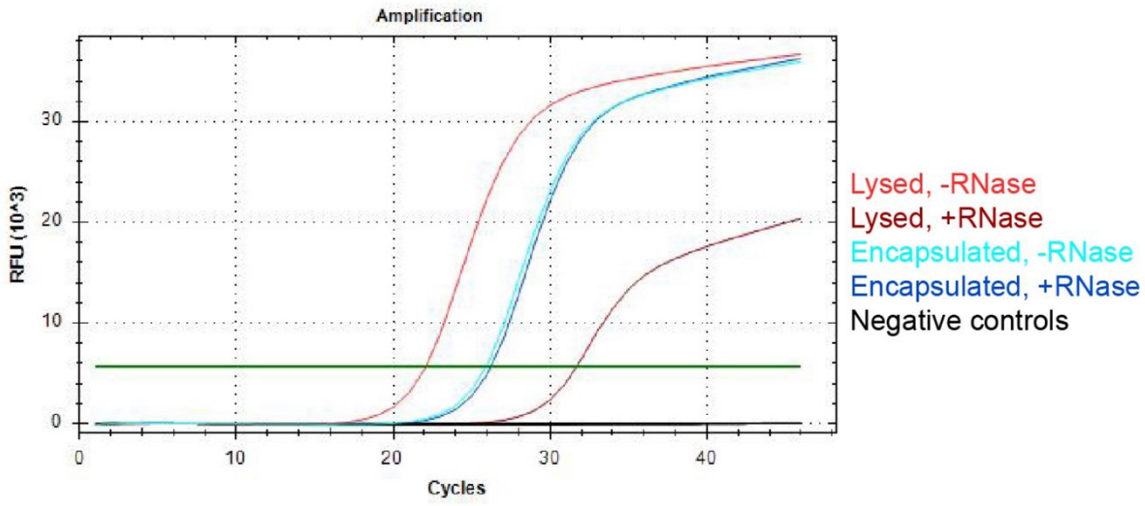

Figure 2: Protection of VariSafe RNA from nuclease degradation in a real-time TaqMan RT-PCR assay.

VariSafe RNAs are easy to lyse: VariSafe RNA can be used directly as template in an amplification reaction or can be lysed by incubation at 65 °C for 5 minutes. Results shown in **Table 1** are for SARS-CoV-2 VariSafe RNA (Catalog #1002) when tested in a real-time RT-PCR assay, both with and without heat lysis. These results show that VariSafe RNAs are easy to lyse.

Table 1: Use of SARS-CoV-2 VariSafe RNA as template in a real-time RT-PCR assay

Template	Unextracted	Heat-lysed for 5 min at		
Target conc. (ng/20 µL rxn.)		65 °C	75 °C	85 °C
	C_t value			
880	14.92	11.17	10.80	12.99
88	16.70	13.54	14.22	14.04
8.8	19.46	16.93	17.84	17.05
0.88	23.73	20.94	21.90	21.12

Direct detection of SARS-CoV-2 in a LAMP assay: VariSafe RNA containing a portion of the SAR-CoV-2 N gene was evaluated for use as a control for development of rapid sample preparation method. A human nasal swab sample was added to 3 mL of viral transport medium (VTM) and spiked with different levels of SARS-CoV-2 VariSafe RNA. The spiked VTM was used as template in an RT-LAMP reaction using Bolt™ Bst polymerase (Varizymes) without RNA extraction. The results presented in **Figure 3** show detection of SARS-CoV-2 in a LAMP assay directly from VTM, both with and without nasal matrix. These results show that VariSafe RNA controls can be used to spike different sample matrices to develop and optimize sample preparation methods.