

Thank you very much for your time today and nice talk. We are really happy we could speak with you and better understand process of this tender. We hope that follow information will be useful for you and please feel free to come back to us if you need more information.

Currently, you are using Thermo one-step kits such as SuperScript tm III Platinum tm One-Step qRT-PCR, or Path-ID Multiplex One-Step RT-PCR. We would like to reassure you that the kits we offer as alternatives are not only compatible with your current workflows, but will also increase your testing capabilities and sensitivity.

The kits that were awarded are One Step PrimeScript™ RT-PCR kit and PrimeScript™ III One Step RT-qPCR mix. Please find below the reasons why our kits won this tender.

1. **Protocols for these kits are extremely similar to your current protocols.** However, thanks to the speed and efficiency of the RT enzyme, the RT step is shorter than most kits at 5 mins.

Please find comparative protocol data below:

One Step PrimeScript™ RT-PCR Kit (Perfect Real Time) shows a similar thermocycler protocol to SuperScript III kit in terms of 2 steps, but includes faster RT and qPCR cycling parameters.

Superscript tm III Platinum tm One-Step qRT-PCR				One Step PrimeScript™ RT-PCR Kit (Perfect Real Time)				
T*C	Time	Stage		T°C	Time	Stage		
		Rev	erse Ti	anscrip	tion			
45-60°C	15-30 min	RT		42°C	5 min	RT		
94°C	2 min	Initial denaturation		95°C	10 sec	Initial denaturation		
			qf	CR				
94°C	15 sec	Denaturation		95°C	5 sec	Denaturation	x45	
55-66°C	30 sec	Anneal	x 40	60°C	30 sec	Hybridation/elongation	X43	
68°C	1 min/kb	Extend						
68°C	5 min	Final extension						

 $Prime Script III \,RT-qPCR \,Mix \,also \,shows \,a \,similar \,thermocycler \,protocol \,to \,Path-ID \,Multiplex \,One-Step \,RT-PCR \,kit, \,but \,also \,with \,faster \,RT \,and \,qPCR \,cycling.$

Path-ID Multiplex One-Step RT-PCR			One Step PrimeScript III RT-qPCR Mix							
T°C	Time	Stage		T°C	Time	Stage				
Reverse Transcription										
48°C	10 min	RT		52°C	5 min	RT				
95°C	10 min	Initial denaturation		95°C	10 sec	Initial denaturation				
				qPCR						
95°C	15 sec	Denaturation	x40	95°C	5 sec	Denaturation	x45			
60°C	45 sec	Hybridation/elongation		60°C	30 sec	Hybridation/elongation				

- 2. Reaction mix preparation is identical between kits: keep the same sample input and primer/probe concentration to what you currently use.
- 3. **PrimeScript III outperforms other RT-qPCR mastermixes**. Brown et al. 2020 (attached) shows that PrimeScript III shows better sensitivity, less false negatives and faster detection compared to Thermo's TaqPath and Fast Virus mixes, and Qiagen's One-Step RT-qPCR mix.
- 4. PrimeScript III is also compatible with samples directly, without RNA extraction: swabs diluted in H_2O (article attached "Brown et al. 2020. Direct RT-qPCR") or **crude lysates from saliva samples** can be used successfully.
- 5. Your Ministry of Health will purchase all kits needed for your testing at a highly negotiated price: One Step PrimeScript RT-PCR kit at (10)(2b) instead of (10)(2b) and PrimeScript III One Step RT-qPCR at (10)(2b) instead of (10)(2b)
- 6. Save time when testing: not only are the PCR cycling protocols themselves faster, but PrimeScript III doesn't freeze at -20° C which means users will not have to thaw the tubes hours before testing.
- 7. Keep the kits as long as needed: PrimeScript III RT-qPCR mix has an **expiry date of 18 months after production** and is stable at 4°C for 2 months once opened.
- 8. Takara Bio Europe can send sample size kits free of charge to all labs that need initial in house validation.

Our solutions will help you save time and energy during these difficult times. Also, we are offering these solutions to your Ministry of Health, Welfare and Sport at a highly discounted price to minimise costs for efficient SARS-CoV-2 testing.

Since the beginning of this outbreak, we have worked with hundreds of laboratories around Europe and the Takara Bio One-Step RT-qPCR enzymes here mentioned have been used in over 20 million SARS-CoV-2 PCR tests in many countries, including the

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We are looking forward to hear from you Best regards

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