BECKMAN COULTER
Life Sciences

Viral RNA Extraction from Saliva and Swab Samples

RNAdvance Viral and RNAdvance Viral XP

The RNAdvance Viral kits are ribonucleic acid (RNA) isolation chemistries built on SPRI paramagnetic bead-based technology. SPRI technology enables purification of high-quality RNA with demonstrated compatibility with up to 200 µL of saliva or swab transport media. The protocol can be performed in a single tube or 96-well format with the flexibility to automate on a variety of liquid handling platforms. Viral RNA extraction begins with lysis of the viral capsid from a variety of sample inputs, including saliva and nasopharyngeal or oropharyngeal swabs. Following lysis, the magnetic beads capture the RNA; washes are then performed to rinse away contaminants including amplification inhibitors.

- Produces high-quality RNA compatible with downstream gene expression analysis techniques, such and qRT-PCR and NGS
- Flexible SPRI technology is amenable to liquid handlers for high-throughput sample processing
- Performance Limit of Detection (LoD) demonstrated at 1 copy/µL

RNAdvance Viral Extraction from Saliva and NP/OP Swab Samples - Analytical Performance

	2019-NCOV_N1		2019-NCOV_N2		2019-NCOV_N3	
RNA Concentration (copies/µL)	1	2	1	2	1	2
No. of positives/ Total No. of replicates	4/4	4/4	4/4	4/4	4/4	4/4
Mean Ct	34.83	35.15	35.17	34.21	35.95	35.48

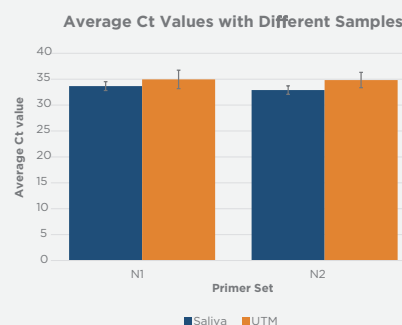**Average Ct Values with Different Samples**

Table 1. (left) RNA was extracted from transport media spiked with Exact Diagnostics SARS-CoV-2 Standard at concentrations of 1 and 2 copies/µL RNA and run in quadruplicates. Ct value was assessed via qRT-PCR for N1, N2 and N3 genes. Both samples containing 1 and 2 copies/µL had comparable Ct values and confirmed 1 copy/µL LoD.

Figure 1. (right) RNA was extracted from 5 saliva samples and 5 Healthlink UTM collection tubes spiked with 1 copy/µL of SARS-CoV-2 isolate and extracted in triplicates. Ct values were assessed by qRT-PCR for N1, N2 and RP (not shown) gene. When comparing saliva and UTM, saliva had lower average Ct values (1 to 2) and less variability with SD of 0.85 and 1.78 for N1 and N2, respectively.

RNAdvance Viral XP Extraction of Transport Media Samples - Analytical Performance

	2019-NCOV_N1			2019-NCOV_N2		
RNA Concentration (copies/uL)	0.33	1	3	0.33	1	3
No. of positives/ Total No: of replicates	16/20	20/20	20/20	14/20	20/20	20/20
Mean Ct	NA*	37.46	35.96	NA*	37.66	36.34

*Mean Ct not calculated due to Not Detectable

Table 2. RNA was extracted from SeraCare positive controls (AccuPlex™ SARS–CoV–2 Reference Material Kit) at concentrations of 0.3, 1 and 3 copies/µL RNA and run in triplicate via RT-PCR. All contrived positive samples above 1 copy/µL were positive, confirming LoD of 1 copy/µL.

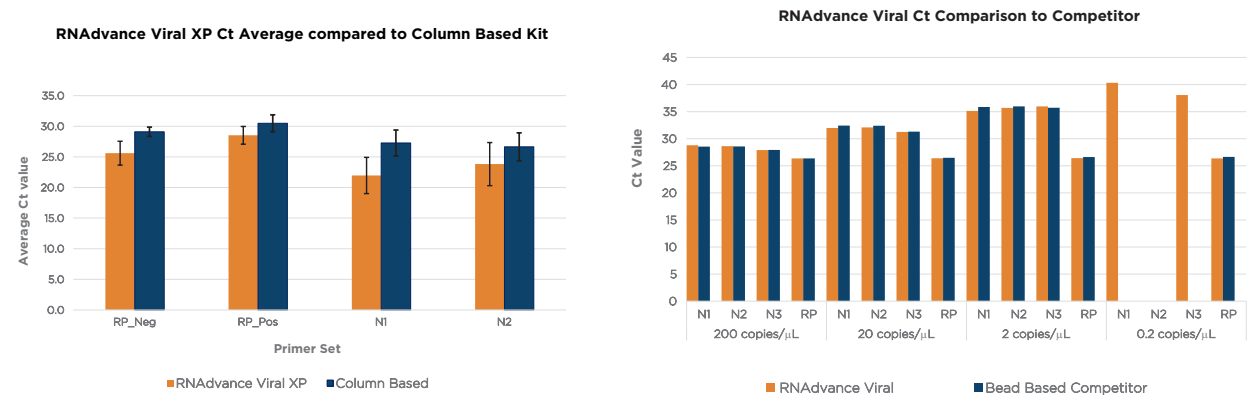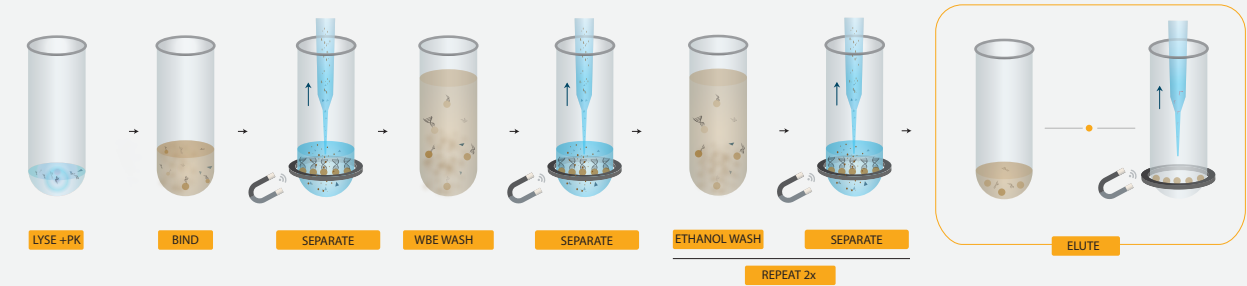## RNAdvance Viral Kits Provide Consistent Performance in downstream RT-PCR workflows

Figure 2. (Left) RNA was extracted from 3 known positive and 3 known negative SARS-CoV-2 samples using RNAdvance Viral XP and Competitor Column Based extraction kit. RT-PCR Ct values were averaged for N1, N2 and RP gene. Error bars represent the standard deviation of the Ct values of the samples. (Right) RNA was extracted manually using RNAdvance Viral or bead-based competitor. Ct values were assessed via qRT-PCR using targeted primer set for SARS-CoV-2 N1, N2, N3 and RP gene and shows comparable Ct values. At 0.2 copy/µL both kits showed Undetermined Ct indicating LoD is 0.2-2 copies/µL.

Median Tissue Culture Infectious Dose ($TCID_{50}$) Study Confirms Inactivation of SARS-CoV-2 with Lysis (LBF)

SARS-CoV-2 viruses was mixed with LBF and incubated under three conditions: at room temperature for 20 min and 60 min, and 60°C for 20 min. The virus-LBF mixture went through filtration and centrifugation to replace LBF with PBS to avoid cell toxicity. The mixture was used to inoculate cells at a 10-fold dilution. The $TCID_{50}$/mL was calculated in Table 3 which indicates that LBF can effectively inactivate SARS-CoV-2 viruses.

Buffer	LBF	LBF	LBF
Temperature	RT	RT	60°C
Time (min)	20	60	20
$TCID_{50}$/mL	$3.16x\ 10^2$	$3.16x\ 10^2$	$3.16x\ 10^2$

Table 3. $TCID_{50}$ assay data. There was no evidence of virus-induced cytopathic effect (CPE) at 10^{-2} dilutions which is equivalent to $3.16x$ 10^2 $TCID_{50}$/mL. Heat inactivation at 60°C for 20 minutes showed the same result.

To further confirm the inactivation of virus, the cells were inoculated with SARS-CoV-2 / LBF lysis mixture. After 5 days in culture, the supernatant was used to inoculate naive cells and $TCID_{50}$ was calculated (10 $TCID_{50}$/mL). The results indicate a greater than 7,940-fold reduction in viral activity, or >99.987% effective viral inactivation with LBF lysis at room temperature for 20 minutes.

Visual Workflow of RNAdvance Viral

1. Lyse tissue in LBF and Proteinase K
2. Bind RNA to magnetic beads
3. Separate magnetic beads from contaminants
4. Wash magnetic beads with WBE
5. Wash magnetic beads with 70% EtOH
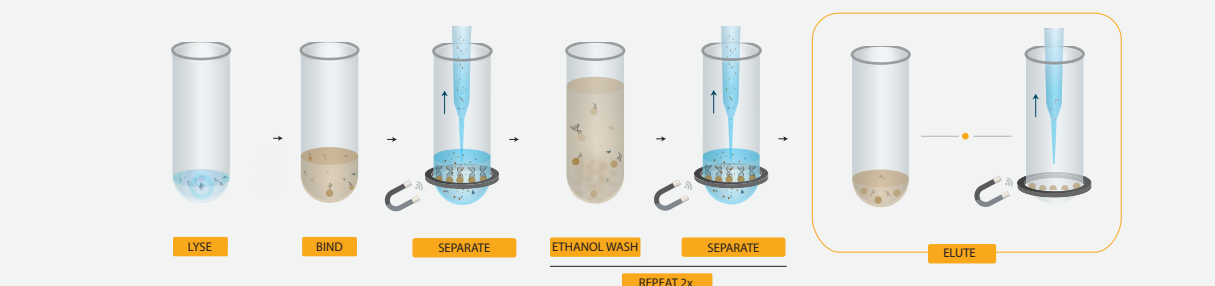6. Elute RNA from magnetic beads
7. Transfer to a new plate

Visual Workflow of RNAdvance Viral XP

1. Lyse sample in Lysis LBF
2. Bind RNA to magnetic beads
3. Separate magnetic beads from contaminants
4. Wash magnetic beads with 70% EtOH
5. Elute RNA from magnetic beads
6. Transfer to a new plate

Extracting RNA from samples can be done efficiently in both manual or automated workflows depending on batch size and overall throughput need
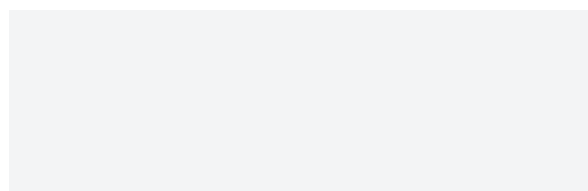
			RNAdvance Viral		RNAdvance Viral XP	
			Manual	Automated	Manual	Automated
Batch	24	Hands-on Time	1 hr, 30 min	15 min	0.25	10 min
		Total Time	2	1 hr, 15 min	0.75	55 min
	96	Hands-on Time	NR	15 min	NR	10 min
		Total Time	NR	1 hr, 15 min	NR	55 min
	192	Hands-on Time	NR	15 min	NR	10 min
		Total Time	NR	1 hr, 30 min	NR	1 hr, 15 min

Table 4. The estimated hands-on time and total time in hours, required to perform 24, 96 and 192 RNAdvance Viral RNA extractions. The methods can be performed either manually or automated on a liquid handling system. Data represented in this table is based on a Biomek i5 Nucleic Acid Solution. NR=Not Recommended.

Product Information

Part No	Name	Preps
C63510	RNAdvance Viral	768
C59543	RNAdvance Viral XP	1056

For more information, please contact:

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at **beckman.com**

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