

Aliquot procedure for Illumina COVIDSeq™ Test (RUO Version) kit reagents

Safe handling and storage for
critical NGS reagents

- Safeguard experimental results with proper reagent handling
- Maximize productivity with aliquot volumes that match experimental workflows
- Preserve reagent effectiveness with best-practice tracking and storage procedures

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Introduction

The Illumina COVIDSeq Test (Catalog no. 20043675 or 20044461) is an amplicon-based next-generation sequencing (NGS) assay that can identify and characterize SARS-CoV-2. The test is designed to help clinical research laboratories identify and track the emergence and prevalence of novel strains of SARS-CoV-2.¹

The COVIDSeq Test kit includes reagents for processing 3072 samples. Not all research laboratories will need to use reagents for all 3072 samples in a single experimental run. This technical note provides guidance for proper aliquoting and storage of COVIDSeq Test kit components into 8 × 384 sample processing sets. These aliquoting suggestions will allow each of the 384-sample aliquots to be freeze-thawed seven times for use with batches containing as few as 54 samples. Other common sample-processing set sizes are 16 × 192 and 32 × 96.

Warnings and precautions

Illumina has defined storage conditions and shelf lives of the reagents included in the kit as indicated in the instruction for use. This aliquot procedure has not been verified or validated for use with the Illumina COVIDSeq Test (RUO Version) kit. It is up to the customer to validate performance of the Illumina COVIDSeq Test (RUO Version) kit if reagents are aliquoted into third-party containers as described.

Before you begin

- Establish a consistent labeling system for all reagent tubes and make sure that all laboratory members understand and adhere to that system
- Include reagent identification, part numbers, and expiry dates in a laboratory reagent tracking system
- Mark expiration dates clearly on each tube
- Make aliquots based on planned sample numbers to minimize reagent selection mistakes
- Organize reagents in separate, clearly labeled containers, or in one-time-use sets, with volumes matching the number of samples intended for each sequencing run
- Follow laboratory best practice first-in, first-out order when using reagents
- Review Tips and Techniques, Avoiding Contamination in the *COVIDSeq Test Reference Guide*²
- Aliquot reagents in a pre-PCR laboratory area using a laminar airflow hood
- Clean all work surfaces using RNaseZap RNase decontamination solution (Thermo Fisher Scientific, Catalog no. AM9782)
- Use positive-displacement pipettes with aerosol-resistant filtered pipette tips
- Use molecular-laboratory grade consumables (storage tubes) certified as RNase/DNase free
- Work quickly when aliquoting frozen reagents to avoid long periods on ice
- Review Tips and Techniques, Handling Beads in the *COVIDSeq Test Reference Guide* prior to making aliquots of bead-containing reagents²
- Maintain storage conditions as indicated on the original stock reagent tubes

Procedure

Recommendations are provided for generating aliquots of COVIDSeq Test (RUO Version) kit reagents into 8 × 384 sample processing sets (Tables 1–4). Storage conditions, suggested volumes, and handling procedures are included. Tables are organized according to Illumina COVIDSeq Test (RUO Version) kit packaging. To prevent contamination that can affect experimental results, some reagents require storage in a cleaned and decontaminated pre- or post-PCR laboratory space as indicated. Handle all reagents carefully and work quickly to minimize the time that reagents spend on ice and not at the recommended storage conditions.

Table 1: COVIDSeq Test Box 1—3072 Samples (Part no. 20044405 or 20044408)

Qty.	Label volume	Reagent	Storage	Fill volume	Aliquot	Suggested aliquot volume	Notes
1	233 ml	Illumina Tune Beads (ITB)	Room temperature	245.3 ml	Optional, to reduce contamination risk	8 × 29 ml	Vortex frequently. Be sure that beads are fully resuspended before making aliquots. Aspirate and dispense slowly due to viscosity of solution. Extra volume included.
1	55.6 ml	Stop Tagment Buffer 2 HT (ST2 HT)	Room temperature	58.4 ml	Optional, to reduce contamination risk	8 × 7 ml	Extra volume included.

Table 2: COVIDSeq Test Box 2—3072 Samples (Part no. 20044406 or 20044409)

Qty.	Label volume	Reagent	Storage	Fill volume	Aliquot	Suggested aliquot volume	Notes
2	6.15 ml	Enrichment BLT HT (EBLTS HT)	2°C to 8°C, post-PCR ^a	6.5 ml × 2 tubes	Optional, to reduce contamination risk	8 × 1.6 ml	Store upright to make sure that beads are always submerged in buffer. Combine two tubes and make sure beads are fully resuspended/thoroughly mixed before making aliquots. Aspirate and dispense slowly due to viscosity of solution. Use tubes large enough to mix the 1.6 ml volume later. Very little extra volume included.
1	10 ml	Resuspension Buffer HT (RSB HT)	2°C to 8°C, post-PCR ^a	10.5 ml	Optional, to reduce contamination risk	8 × 1.25 ml	Extra volume included.
1	114 ml	Elution Buffer HT (ELB HT)	2°C to 8°C, pre-PCR ^b	120 ml	Optional, to reduce contamination risk	8 × 14 ml	Extra volume included.
1	845 ml	Tagmentation Wash Buffer HT (TWB HT)	2°C to 8°C, post-PCR ^a	887.4 ml	Optional, to reduce contamination risk	8 × 105 ml	Extra volume included.

a. Store in a properly cleaned and decontaminated post-PCR lab space

b. Store in a separate cleaned and decontaminated pre-PCR lab space

Table 3: COVIDSeq Test Box 3—3072 Samples (Part no. 20044407 or 20044410)

Qty.	Label volume	Reagent	Storage	Fill volume	Aliquot	Suggested aliquot volume	Notes
1	45.1 ml	Elution Prime Fragment 3HC Mix HT (EPH3 HT)	-25°C to -15°C, pre-PCR ^a	47.4 ml	Yes	8 × 5.5 ml	Thaw at room temperature and invert to mix before use. Extra volume included.
1	100.6 ml	Illumina PCR Mix HT (IPM HT)	-25°C to -15°C, pre-PCR ^a	105.6 ml	Yes	8 × 12.5 ml	Thaw at room temperature and invert to mix before use. Inspect for precipitates. If present, vortex tube until crystals dissolve. Keep on ice during use. Extra volume included.
1	78.9 ml	Enhanced PCR Mix HT (EPM HT)	-25°C to -15°C, pre-PCR ^a	83 ml	Yes	8 × 10 ml	Invert to mix. Keep on ice during use. Extra volume included.
1	4.6 ml	Reverse Transcriptase HT (RVT HT)	-25°C to -15°C, pre-PCR ^a	5.1 ml	Yes	8 × 600 µl	Invert to mix. Keep on ice during use. Extra volume included.
1	41.1 ml	First Strand Mix HT (FSM HT)	-25°C to -15°C, pre-PCR ^a	43.2 ml	Yes	8 × 5 ml	Thaw at room temperature and invert to mix. Keep on ice before use. Extra volume included.
1	14.4 ml	COVIDSeq Primer Pool 1 HT (CPP1 HT)	-25°C to -15°C, pre-PCR ^a	15.2 ml	Yes	8 × 1.8 ml	Thaw at room temperature. Invert to mix. Keep on ice during use.
1	14.4 ml	COVIDSeq Primer Pool 2 HT (CPP2 HT)	-25°C to -15°C, pre-PCR ^a	15.2 ml	Yes	8 × 1.8 ml	Thaw at room temperature. Invert to mix. Keep on ice during use.
1	37.6 ml	Tagmentation Buffer 1 HT (TB1 HT)	-25°C to -15°C, post-PCR ^b	39.5 ml	Yes	8 × 4.7 ml	Bring to room temperature. Vortex thoroughly before use.

a. Store in a separate cleaned and decontaminated pre-PCR lab space
 b. Store in a properly cleaned and decontaminated post-PCR lab space

Table 4: COVIDSeq Positive Control HT—3072 Samples
(Part no. 20043401 or 20044883)

Qty.	Label volume	Reagent	Storage	Fill volume	Aliquot	Suggested aliquot volume	Notes
1	100 µl	COVIDSeq Positive Control HT (CPC HT)	-85°C to -65°C, pre-PCR ^a	not listed	Yes	8 × 10 µl	Critical storage temperature noted. CPC HT reagent should be thawed no more than two times. Aliquot unused CPC HT reagent at the first thaw and store at the indicated storage temperature for future use. First dilution of CPC HT can be stored at the indicated storage temperature for future use. Use low bind tubes. Keep on ice during use.

a. Store in a separate cleaned and decontaminated pre-PCR lab space

References

1. Illumina Inc. COVIDSeq Test (RUO Version). Illumina.com. [illumina.com/products/by-type/clinical-research-products/covidseq.html](https://www.illumina.com/products/by-type/clinical-research-products/covidseq.html). Accessed March 10, 2021.
2. Illumina Inc. Illumina COVIDSeq Test Reference Guide. Illumina.com. https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/Illumina-COVIDSeq-Test/illumina-covidseq-test-reference-guide-ruo-1000000126053-04.pdf. Accessed May 11, 2021.



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