Crescendo cDNA Synthesis for qPCR.

PRODUCT SHEET

Robust solution for enhanced qPCR detection from low input and degraded samples

The Crescendo cDNA Synthesis for qPCR system is designed to efficiently convert and amplify RNA in order to generate large quantities of cDNA. Using our robust Single Primer Isothermal Amplification (SPIA®) technology, over a microgram of cDNA can be generated from as little as 500 pg of total RNA. This kit is compatible with inputs as low as 500 pg and degraded samples allowing you to study a broad range of sample types. The resulting cDNA can be used for qPCR or archiving.

Why use the Crescendo cDNA System for qPCR?

1. **Increased sensitivity:** Amplified cDNA increases the number of target copies, offering accurate detection by qPCR
2. **Compatible with degraded samples:** SPIA amplification is compatible with limited and degraded RNA allowing analysis of previously inaccessible samples
3. **Robust amplification:** Highly published, robust technology provides a simple solution for sensitive qPCR
4. **Application flexibility:** Generates micrograms of cDNA to provide material for a range of applications and archiving

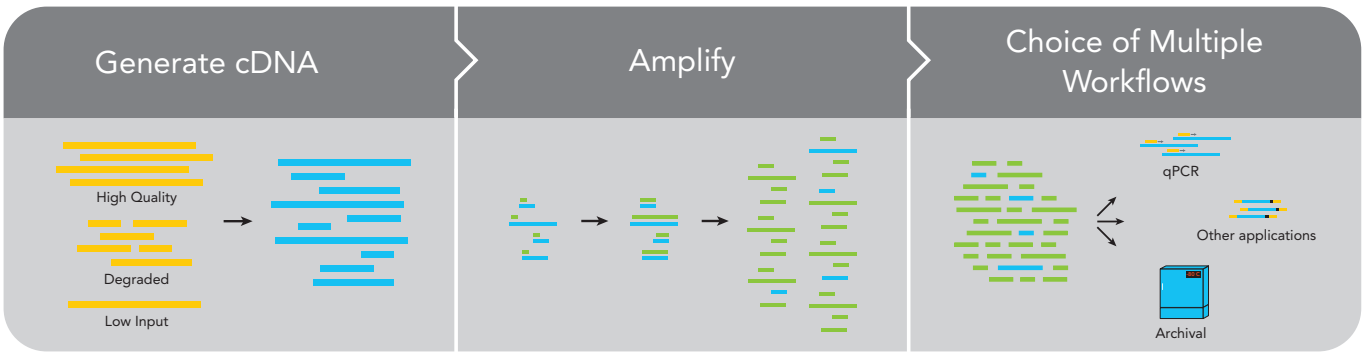

Figure 1: The Crescendo cDNA Synthesis for qPCR kit provides a simple workflow to convert and amplify low input and poor quality total RNA to abundant cDNA suitable for qPCR or other applications.

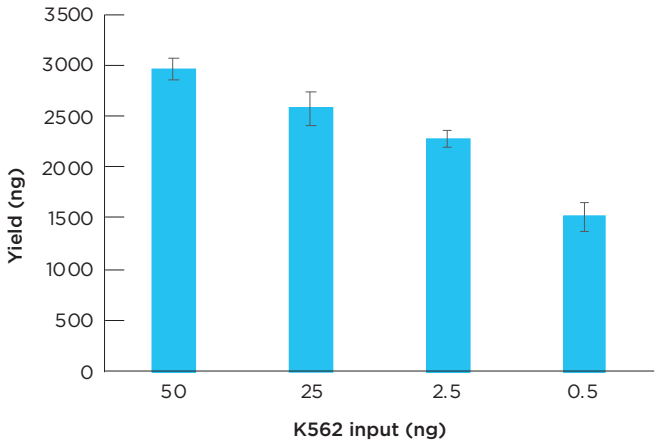

Figure 2: Crescendo cDNA Synthesis for qPCR provides significant amplification of your sample across a broad input range. The yield of cDNA generated is proportional to the input amount and RNA quality. Even at 500 pg of K562 total RNA, over a microgram of cDNA is generated providing sufficient cDNA for all your experimental needs.

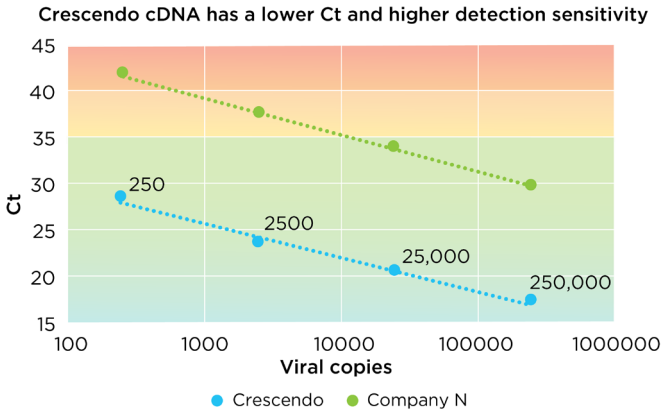

Figure 3: Amplified cDNA provides better detection of SARS-CoV-2. A 10-fold dilution series of SARS-CoV-2 RNA, ranging from 250 to 250,000 copies was added to a background of 2.5 ng of K562 total RNA. A first strand cDNA synthesis kit from Company N was used to generate first strand cDNA, while the Crescendo cDNA Synthesis for qPCR kit was used to convert and amplify each sample to micrograms of cDNA. The CDC SARS-CoV-2_N2 TaqMan™ assay was used to determine the sensitivity of detection from both cDNA synthesis methods. The SARS-CoV-2 RNA was detected significantly earlier in the amplified cDNA sample compared to first strand synthesis alone. For example, the sample containing 250 SARS-CoV-2 copies was easily detected in the Crescendo amplified cDNA (Ct = 28.4) within 35 PCR cycles (shaded green) compared to the first strand synthesis method (Ct = 41.9) which required significantly more PCR cycles.

The Crescendo cDNA Synthesis for qPCR kit provides a simple method for whole transcriptome conversion of between 500 pg and 50 ng of total RNA into double stranded cDNA. Robust amplification provides over a microgram of cDNA even when starting with just 500 pg of high quality RNA (Figure 2). The performance of cDNA amplified by the Crescendo cDNA Synthesis for qPCR kit is compared to standard first strand cDNA synthesis in Figure 3.

Across a range of SARS-CoV-2 RNA spike-ins, the amplified cDNA consistently provided lower Ct values compared to first strand synthesis alone. At 250 viral copies, the Crescendo cDNA Synthesis for qPCR workflow detected SARS-CoV-2 with a Ct of 28.4, while the first strand synthesis workflow required over 40 PCR cycles to detect the virus. Higher Ct values risk the possibility of interference from background PCR amplification or false negative results. Finally, the slopes for the two cDNA conversion methods are similar indicating that the cDNA amplification did not alter the viral titer levels. The amplified cDNA from the Crescendo cDNA Synthesis for qPCR kit is ideal for qPCR studies, whether it is just a few assays or 384- well qPCR array while still retaining sufficient cDNA for archiving your precious samples.

Features

- Reliable cDNA conversion from limited and poor quality total RNA
- Broad input range from 500 pg to 50 ng allows access to any sample
- Generate micrograms of cDNA for downstream applications

Applications

- TaqMan or SYBR® Green qPCR
- Archiving

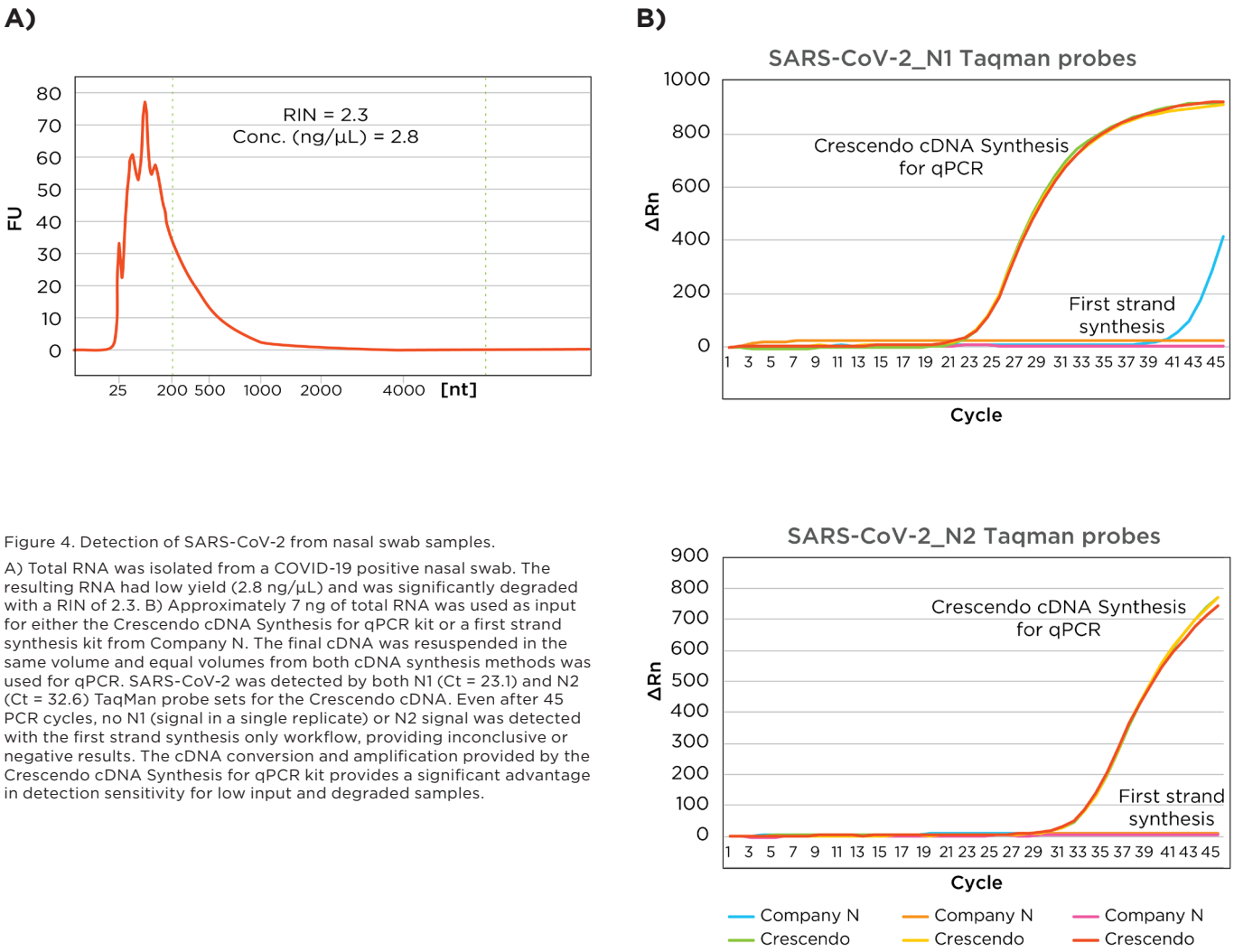

Figure 4. Detection of SARS-CoV-2 from nasal swab samples.

A) Total RNA was isolated from a COVID-19 positive nasal swab. The resulting RNA had low yield (2.8 ng/µL) and was significantly degraded with a RIN of 2.3. B) Approximately 7 ng of total RNA was used as input for either the Crescendo cDNA Synthesis for qPCR kit or a first strand synthesis kit from Company N. The final cDNA was resuspended in the same volume and equal volumes from both cDNA synthesis methods was used for qPCR. SARS-CoV-2 was detected by both N1 (Ct = 23.1) and N2 (Ct = 32.6) TaqMan probe sets for the Crescendo cDNA. Even after 45 PCR cycles, no N1 (signal in a single replicate) or N2 signal was detected with the first strand synthesis only workflow, providing inconclusive or negative results. The cDNA conversion and amplification provided by the Crescendo cDNA Synthesis for qPCR kit provides a significant advantage in detection sensitivity for low input and degraded samples.

Ordering information

Product Name	Part no.	No. of reactions
Crescendo cDNA Synthesis for qPCR	30183903	16
Crescendo cDNA Synthesis for qPCR	30183905	64

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