Promega

Product Application

Direct Viral RNA Detection from XpressAmp™ Lysates

Use RT-qPCR to detect viral RNA in viral transport medium (VTM) from lysates created using the XpressAmp™ Direct Amplification Reagents, Custom Kit.

Kit: XpressAmp™ Direct Amplification Reagents, Custom (Cat.# AX8880)

Analyses: RT-qPCR

Sample Type(s): Samples collected in viral transport medium (VTM[1]), e.g. nasopharyngeal swabs

Input: 5µl lysate

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information contact Technical Services at: **techserv@promega.com**

Materials Required:

- XpressAmp™ Direct Amplification Reagents, Custom (Cat.# AX8880)
- GoTaq® Probe 1-Step RT-qPCR System (Cat.# A6120)

Protocol:

1. Prepare XpressAmp™ Lysis Buffer, Custom with 1% (v/v) 1-Thioglycerol. For example, add 2µl 1-Thioglycerol + 198µl XpressAmp™ Lysis Buffer, Custom.
2. Prepare sample lysates by combining the VTM sample 1:1 with XpressAmp™ Lysis Buffer, Custom with 1% 1-Thioglycerol. For example, add 2.5µl VTM + 2.5µl buffer. Pipette to mix.
3. Incubate sample lysates at room temperature for 10 minutes.
4. Detect viral RNA in sample lysates using RT-qPCR.
   - The reaction should contain XpressAmp™ Solution at an equal volume as the sample lysate volume in the reaction. In the example using GoTaq® Probe 1-Step RT-qPCR System below, 5µl of the sample lysate and 5µl of XpressAmp™ Solution are added to the RT-qPCR reaction.

Component	Volume per Reaction
GoTaq® Probe qPCR Master Mix, 2X	12.5µl
GoScript™ RT Mix for 1-Step RT-qPCR, 50X	0.5µl
25X Primer-Probe Mix*	1µl
XpressAmp™ Solution	5µl
Nuclease-Free Water*	1µl
Sample Lysate	5µl
Final Reaction Volume	**25µl**

**The volume of Primer-Probe Mix and Nuclease-Free Water can be adjusted appropriately to accommodate different Primer-Probe mixes.*

Results:

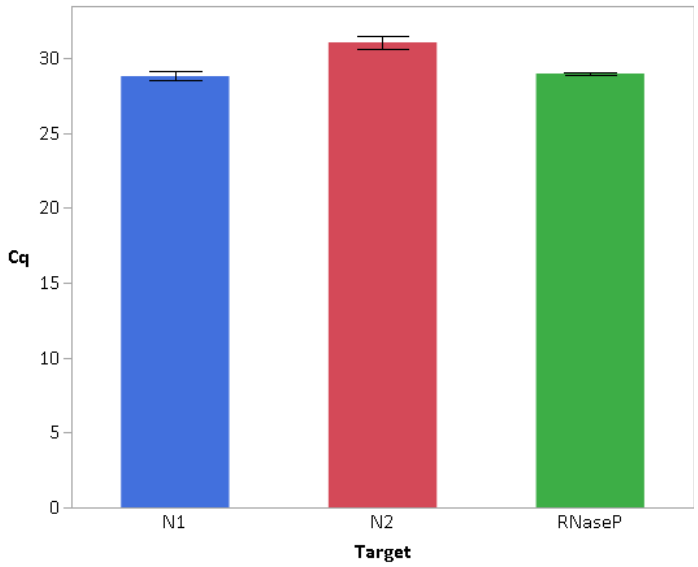

Figure 1. Amplification of synthetic SARS-CoV-2 RNA from XpressAmp™ lysates. VTM was inoculated with a nasopharyngeal swab and spiked with $1x10^4$ copies/µl of Synthetic SARS-CoV-2 RNA Control 2 (Twist Biosciences, Cat.# 102024). XpressAmp™ lysates were prepared from spiked VTM sample as described above. 25µl singleplex GoTaq® Probe 1-step RT-qPCR System (Cat.# A6121) reactions supplemented with XpressAmp™ Solution were assembled using the 2019 nCoV RUO Kit (IDT, Cat.# 10006713) primers and probes and 5µl XpressAmp™ lysates. Reactions were subjected to thermal cycling according to the CDC protocol[2] for GoTaq® Probe 1-step RT-qPCR System. N=8 amplification replicates.

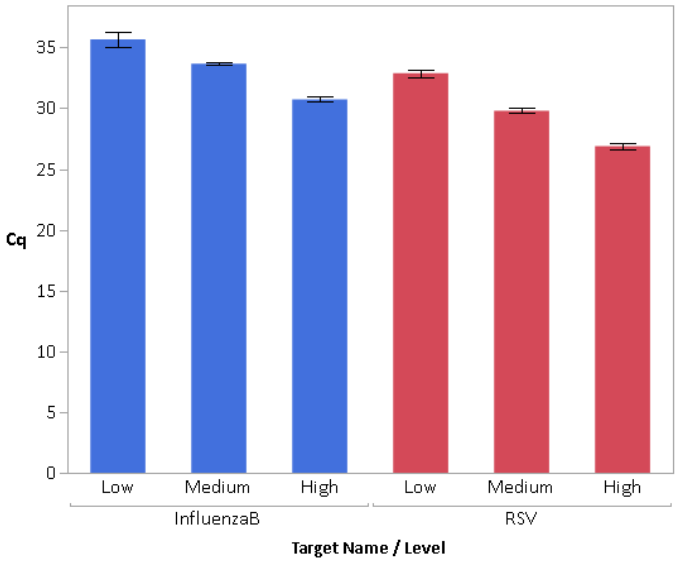

Figure 2. Amplification of Influenza B and RSV A RNA from XpressAmp™ lysates. VTM was inoculated with RSV A and Influenza B (Hong Kong) virus reconstituted from Helix Elite™ Inactivated Standard Influenza A/B and Respiratory Syncytial Virus (Microbiologics Cat.# HE0044N). This high virus sample ($1x10^3$ copies/µl) was diluted 1:10 and 1:100 in VTM to create the medium and low virus level samples. XpressAmp™ lysates were prepared from spiked VTM sample as described above. 25µl singleplex GoTaq® Probe 1-step RT-qPCR system (Cat.# A6121) reactions supplemented with XpressAmp™ Solution were assembled using viral specific primers and probes and 5µl XpressAmp™ lysates. The presence of RSV A and Influenza B was detected by RT-qPCR using GoTaq® 1-Step Probe qPCR System. N=8 amplification replicates.

Reference:

1. Centers for Disease Control and Prevention. (2020). Preparation of Viral Transport Medium. SOP# DSR-052-01. Retrieved from https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf.

2. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel EUA. CDC-006-00019, Revision: 05, Effective 07/13/2020.