TECHNICAL MANUAL

XpressAmp™ Direct Amplification Reagents

Instructions for Use of Products
A8880 and A8882

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TM647

XpressAmp™ Direct Amplification Reagents

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The XpressAmp™ Direct Amplification Reagents contain components for performing fast, extraction-free preparation of viral samples for PCR-based amplification using commonly available PCR reagents from samples collected by nasopharyngeal swab in universal or viral transport medium. The reagents allow the user to perform direct amplification analysis in RT-qPCR following a 10-minute room-temperature incubation that is easy to automate.

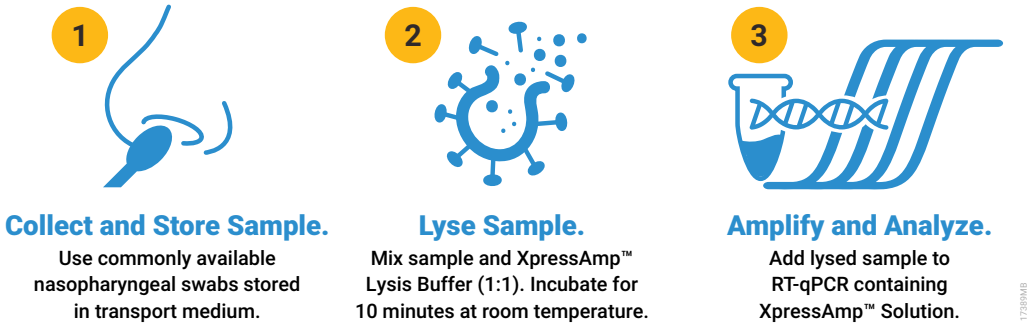

Figure 1. Overview of XpressAmp™ direct amplification workflow.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
XpressAmp™ Direct Amplification Reagents	**250 reactions**	**A8882**

For Laboratory Use. Contains sufficient reagents for manually preparing 250 × 25µl amplification reactions. Includes:

- 15ml XpressAmp™ Lysis Buffer
- 2 × 75µl 1-Thioglycerol
- 1.5ml XpressAmp™ Solution

PRODUCT	SIZE	CAT.#
XpressAmp™ Direct Amplification Reagents, HT	**3,000 reactions**	**A8880**

For Laboratory Use. Contains sufficient reagents for manually preparing 3,000 × 25µl amplification reactions. This product size contains additional volumes of XpressAmp™ Lysis Buffer and 1-Thioglycerol to accommodate high-throughput (HT) sample preparation using common automated liquid handling instrumentation. Includes:

- 200ml XpressAmp™ Lysis Buffer
- 2 × 1ml 1-Thioglycerol
- 18ml XpressAmp™ Solution

Storage Conditions: Upon receipt, remove and store the XpressAmp™ Lysis Buffer at +15°C to +30°C. Store the 1-Thioglycerol and XpressAmp™ Solution at +2°C to +10°C.

Safety Information:

The XpressAmp™ Direct Amplification Reagents are designed to be used with potentially infectious substances. Wear appropriate personal protective equipment when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.

1-Thioglycerol is toxic. Wear gloves and follow standard safety procedures while working with this solution.

3. Materials to Be Supplied by the User

- sterile, aerosol-resistant pipette tips
- vortex mixer
- tubes to prepare solutions, sample lysate and amplification reaction master mix
- real-time RT-qPCR reagents (e.g., GoTaq® Probe 1-Step RT-qPCR System [Cat.# A6120, A6121])
- qPCR primer-probe mix (at least 25X concentration)
- real-time PCR instrument and related consumables (i.e., optical-grade PCR plates and plate seals)

4. Preparing Solutions

1. Ensure the XpressAmp™ Lysis Buffer is at room temperature (15–30°C) before use. If the XpressAmp™ Lysis Buffer has been stored below room temperature, you may see a precipitate. Warm the XpressAmp™ Lysis Buffer to room temperature, with gentle mixing, for at least 30 minutes prior to use to ensure the precipitate is back in solution.
2. Add 1-Thioglycerol to a concentration of 1% (v/v) to an aliquot of XpressAmp™ Lysis Buffer before use.

   Note: XpressAmp™ Lysis Buffer containing 1% 1-Thioglycerol can be used for up to 8 hours after preparation. For best results, use XpressAmp™ Lysis Buffer containing 1% 1-Thioglycerol within 1 hour of preparation.

Component	Volume for 1 Amplification Reaction	Volume for 96 Amplifications Reactions
1-Thioglycerol	0.025µl	2.5µl
XpressAmp™ Lysis Buffer	2.475µl	247.5µl
Total Volume	2.5µl	250µl

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5. Preparing XpressAmp™ Sample Lysate from Universal Transport Medium (UTM) or Viral Transport Medium (VTM) Samples Inoculated with Nasopharyngeal Swabs

1. For each amplification reaction of a VTM/UTM sample (1), combine the VTM/UTM sample 1:1 with freshly prepared XpressAmp™ Lysis Buffer containing 1% 1-Thioglycerol. If performing multiple amplifications from a single VTM or UTM sample, you can prepare a larger sample lysate.

Sample may be potentially infectious. Use appropriate protective equipment when handling samples. Adhere to your institutional guidelines for the handling and disposal of potentially infectious substances when using these reagents.

Component	Volume for Single Amplification Reaction/ Sample	Volume for Multiple (X) Amplification Reactions/ Sample
VTM or UTM Sample[1]	2.5µl	2.5µl × (X + 1) reactions
Prepared XpressAmp™ Lysis Buffer containing 1% 1-Thioglycerol	2.5µl	2.5µl × (X + 1) reactions
Total Volume	5µl	5µl × (X + 1) reactions

[1]XpressAmp™ Direct Amplification Reagents are not compatible with all transport medium that contains guanidinium thiocyanate.

2. Mix the samples by pipetting.
3. Incubate at room temperature for 10 minutes.
4. Proceed to PCR amplification of the XpressAmp™ sample lysate. Holding XpressAmp™ sample lysates for greater than 24 hours before RT-qPCR amplification reduces amplification sensitivity.

6. Guidelines for Amplifying XpressAmp™ Sample Lysates

6.A. General Considerations

Important:

- Add the XpressAmp™ Solution to the PCR master mix. **Do not** add XpressAmp™ Solution directly to prepared sample lysate.
- Failure to add the XpressAmp™ Solution to the PCR master mix will result in poor amplification performance.
- **Do not** use transport medium containing guanidinium thiocyanate. Samples containing this reagent are incompatible with the XpressAmp™ Direct Amplification Reagents and will not amplify.

Using the XpressAmp™ sample lysate and XpressAmp™ Solution in a PCR assay may require re-optimization of the thermal cycling parameters used for a previously optimized PCR assay. Perform an experiment to evaluate reaction annealing temperatures in 2°C, 4°C, and 6°C increments to optimize PCR amplification assays containing XpressAmp™ sample lysate and XpressAmp™ Solution.

6.B. Example RT-qPCR Amplification Using the GoTaq® Probe 1-Step RT-qPCR System

1. Determine the number of reactions to be set up, including control reactions. Prepare enough amplification reaction mix for the number of reactions needed plus 10% additional reaction volume to compensate for pipetting error. This ensures that you have enough reaction mix for all samples.

2. Prepare the amplification reaction mix (minus the sample lysate) by combining qPCR master mix, reverse transcription (RT) mix, primer/probe mix, XpressAmp™ Solution and Nuclease-Free Water. The example below shows how to set up the amplification reaction mix when using the GoTaq® Probe 1-Step RT-qPCR System. Vortex briefly to mix. The prepared XpressAmp™ sample lysate is added in Step 4.

Component	Starting Concentration	Reaction Final Concentration	Single-Well Reaction Volume	Number of Reactions (n × 1.1)	Master Mix Volume
GoTaq® Probe qPCR Master Mix with dUTP (2X)[1,2]	2X	1X	12.5µl	×	=
GoScript™ RT Mix for 1-Step RT-qPCR	50X	1X	0.5µl	×	=
Primer/Probe Mix[3]	25X	Primers: 0.1–1µM Probe: 0.1–0.5µM	1µl	×	=
XpressAmp™ Solution[3,4]	5X	1X	5µl	×	=
Nuclease-Free Water			1µl	×	=
Prepared XpressAmp™ sample lysate[3]			5µl		
Final Reaction Volume			25µl		

[1]If using the GoTaq® Probe 1-Step RT-qPCR System, consult the *GoTaq® Probe 1-Step RT-qPCR Technical Manual #*TM379 to determine whether CXR reference dye is required for your instrument and for instructions on how to add the dye to your reaction mix.

[2]XpressAmp™ Direct Amplification Reagents are compatible with other commercially available RT-qPCR and qPCR master mixes. For guidance on using these reagents with other amplification mixes, please contact Promega Technical Services.

[3]If adapting a previously optimized PCR assay to direct amplification by adding XpressAmp™ Solution to your reaction, you may need to reoptimize your PCR amplification conditions (i.e., annealing temperature and primer/probe concentration).

[4]You can change the final volume of the PCR amplification to accommodate other volumes of prepared XpressAmp™ sample lysate. If you change the final PCR volume, adjust the volume of XpressAmp™ Solution to ensure a 1X final reaction concentration.

3. Add 20µl of reaction mix (without XpressAmp™ sample lysate) to each PCR tube or well of an optical-grade PCR plate.

4. Add 5µl of XpressAmp™ sample lysate to the appropriate tubes or wells of the reaction plate.

6.B. Example RT-qPCR Amplification Using the GoTaq® Probe 1-Step RT-qPCR System (continued)

5. Seal the tubes or optical plate. Centrifuge briefly to collect the contents at the bottom of tubes or wells. The samples are ready for thermal cycling. Protect tubes or plate from extended light exposure or elevated temperatures before cycling.

6. Begin thermal cycling. The cycling parameters in the table below are provided as a guideline.*

	Example Cycling Conditions		
Step	**Cycles**	**Temperature**	**Time**
Reverse transcription	1	45°C	15 minutes
Reverse transcriptase inactivation/ GoTaq® polymerization activation	1	95°C	2 minutes
Denaturation	45	95°C	3 seconds
Annealing/extention*		55°C	30 seconds

*The optimal annealing and extension temperatures and times are dependent on the sequence of your primers/probes, the length of your amplicon, and the amplification reagents used in the RT-qPCR. We recommend optimizing the annealing/extension temperature and time for your XpressAmp™ Direct Amplification RT-qPCR assay before testing samples.

7. Reference

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

8. Related Products

Product	Size	Cat. #
GoTaq® Probe 1-Step RT-qPCR System	2ml	A6120
	12.5ml	A6121