Lyo-Ready Direct RNA NASBA Stool

Product Handling Guide

Shipping:	Blue Ice
Catalog number:	MDX222
Batch No.:	See vial
Concentration:	2.5x

Store at –20 °C

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Storage and stability:
Lyo-Ready Direct RNA NASBA Stool is shipped on blue ice. On arrival store at -20 °C for optimum stability. Repeated freeze/thaw cycles should be avoided. Solutions should be mixed/equilibrated after each thawing to avoid phasing.

Expiry:
When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

Safety precautions:
Read and understand the SDS (Safety Data Sheets) before handling the reagents. Hardcopies of the SDS will be provided with the first shipment, thereafter they will be available upon request.

Quality control:
Meridian operates under ISO 13485 Quality Management System. Lyo-Ready Direct RNA NASBA Stool and its components are extensively tested for activity, efficiency, absence of nuclease contamination and absence of nucleic acid contamination.

Notes:
For research and further manufacturing use only.

Description

Lyo-Ready Direct RNA NASBA Stool is a glycerol-free mix for the isothermal amplification technique Nucleic Acid Sequence-Based Amplification (NASBA). It contains enzymes (reverse transcriptase, RNA polymerase and RNase H), reaction buffer, $MgCl_2$, nucleoside triphosphates (NTPs/dNTPs) and excipients allowing ambient temperature stabilization of assays through lyophilization. Lyo-Ready Direct RNA NASBA Stool has been designed for highly reproducible, accurate RNA target amplification, delivering excellent results even in the presence of crude gastrointestinal samples. In order to produce room temperature lyophilized NASBA reagents, assay specific primers and molecular beacons can be added to Lyo-Ready Direct RNA NASBA Stool for subsequent lyophilization.

Kit components

Table 1

Component
Lyo-Ready Direct RNA NASBA Stool, 2.5x

Users Guidelines

Before you start:

- **Optimal length of NASBA primers is 20-25 nt. One primer for NASBA must include in addition the promoter sequence for T7 RNA polymerase at the 5' end: (5'-AATTCTAATACGACTCACTATAGGG-).**
- **Amplicon length should be 100–250 bp**
- **The amount of inhibition tolerated by Lyo-Ready Direct RNA NASBA Stool is variable depending on several factors, including assay design and sample quality. For this reason, an initial sample titration is recommended.**
- **It is recommended to include a no-template control (NTC) to verify product specificity.**
- **Prior to use or storing at -20 °C, the thawed reagents must be thoroughly mixed by briefly vortexing.**

Suggested 1-step NASBA reaction conditions:

The following protocol is for a standard 20 µL NASBA reaction to be used as a starting point for optimization.

Assemble the reaction mixture in ice as indicated in table 2.

Table 2

Reagent	Volume	Final concentration
Lyo-Ready Direct RNA NASBA Stool, 2.5x	8 µL	1x
Primer-Beacon Mix, 20x*	1 µL	1x
DMSO	3 µL	15 %
Sample RNA	variable	
Water (ddH_2O)	to 20 µL	

** Primers and molecular beacon concentration and ratio need be optimized*

Vortex thoroughly, pulse-spin and incubate at 41 °C for 60 minutes.

Master mix preparation for lyophilization

Assemble the reaction in a microcentrifuge tube on ice as indicated in table 3. The volumes shown are for a 20 µL reaction.

Table 3

Reagent	Volume	Working concentration
Lyo-Ready Direct RNA NASBA Stool, 2.5x	8µL	2x
Primer-Beacon Mix, 20x*	1 µL	2x
Water (ddH_2O)	to 10 µL	

** Primers and molecular beacon concentration and ratio need be optimized*

For preparation of lyo-cakes, dispense into reaction vials, such as PCR strip tubes or plates, and immediately pre-freeze the vials at –80°C before transferring into freeze-dryer.

For preparation of lyo-beads, dispense the liquid master mix into liquid nitrogen and immediately transfer into freeze-dryer.

Run a suitable lyophilization cycle (See: Lyophilization and Post-lyophilization User Guideline).

Seal lyophilized material with a silica sachet in a heat sealed pouch at low relative humidity conditions and store at room temperature until ready for use.

Rehydrate the lyophilized NASBA master mix in the reaction vials with 17 µL template-containing solution and 3 µL of DMSO. Vortex thoroughly, pulse-spin and incubate at 41 °C for 60 minutes.

Associated Products

Component	Cat. No.
Lyo-Ready Direct RNA NASBA Blood, 2.5x	MDX220
Lyo-Ready Direct RNA NASBA Saliva, 2.5x	MDX221
Lyo-Ready Direct RNA NASBA Urine, 2.5x	MDX223

Technical support

For any technical enquiries, please contact our Technical Support team via email at: mbi.tech@meridianlifescience.com

Meridian Life Science Inc.
USA
Tel: +1 901 382 8716
Fax: +1 901382 0027

Lyophilization & Post-Lyophilization User Guideline

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The guidelines in this document can help users avoid problems in lyophilization for lyo-bead production. For storage and stability, expiry and general handling of these product pre-lyophilization, please refer to the individual Product Handling Guides.

Safety precautions:

Read and understand the SDS (Safety Data Sheets) before handling the reagents. Copies of these SDSs are available on our website or upon request.

There are several advantages for lyophilization, including room temperature shipping and storage, extended shelf-life and increased flexibility in sample volume. In order to be compatible with lyophilization however, enzyme preparations must be glycerol-free and include specialized lyophilization-excipients that preserve the mixture as it is exposed to various lyophilization conditions including freezing, temperature ramps, vacuum and dehydration. An ideal lyophilization formulation should stabilize an enzyme in a freeze-dried format and allow very fast rehydration and reactivation of the enzyme preparations, without impacting its performance post rehydration.

Lyophilization

- The lyophilization cycle protocols in Table 1 is suitable for lyophilization of Lyo-Ready NASBA mixes in lyophilized cake or bead format. These parameters are provided as a guidance only and should be optimized to different user formats and systems.
- The product already contains excipients, therefore, there should be no need to add any further excipients to assist lyophilization.

Table 1. Lyophilization guidelines

Step	Temperature	Time	Description	Vacuum Set Points
Shelf Pre-Freezing	-40°C	n/a	Pre-Freezing	
Freezing	-45 °C	0.5 °C/min	Ramp	
	-45 °C	10 min	Hold	
Evacuation	-55 °C (Condenser)			75 mTorr (100 µbar)
Primary Drying*	-45 °C	180 min	Hold	37.5 mTorr (50 µbar)
	-40 °C	0.5 °C/min	Ramp	
	-40 °C	900 min	Hold	
Secondary Drying	+22 °C	0.5 °C/min	Ramp	37.5 mTorr (50 µbar)
+22 °C	240 min			Hold

**Primary drying time could be extended to up to 24h depending on quantity of sample that is lyophilized*

Post-Lyophilization

- Lyophilized NASBA mixes must be handled in a humidity-controlled environment of <5% humidity to ensure storage stability.
- For maximum shelf-life, we suggest packaging lyophilized material under inert gas conditions (e.g. nitrogen or argon) and insert a desiccant sachet to improve stability.
- Pouches should be heat-sealed and labelled.