

Liquid Biopsy Stool DNA qPCR Mix

Product Handling Guide

Shipping:	Blue Ice
Catalog number:	ODX005
Batch No.:	See vial
Concentration:	4x

Store at **-20 °C**



Storage and stability:

Liquid Biopsy Stool DNA qPCR Mix is shipped on blue ice. On arrival store at -20 °C for optimum stability. Repeated freeze/thaw cycles should be avoided. Thawing during transportation does not affect the product performance. 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. Liquid Biopsy Stool DNA qPCR Mix and its components are extensively tested for activity, efficiency, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

Notes:

For research and further manufacturing use only.

Description

Liquid Biopsy Stool DNA qPCR Mix combines the latest advances in buffer chemistry and PCR enhancers, together with an optimized concentration of antibody-mediated hot-start polymerase, dNTPs and MgCl₂. Liquid Biopsy Stool DNA qPCR Mix has been designed for highly reproducible, accurate DNA target amplification, delivering excellent results in multiplex assays, even in the presence of inhibitors present in stool samples.

Kit components

Component

Liquid Biopsy Stool DNA qPCR Mix

General considerations

To help prevent any carry-over DNA contamination, we recommend that separate areas are maintained for reaction set-up, PCR amplification and any post-PCR gel analysis. It is essential that any tubes containing amplified PCR product are not opened in the PCR set-up area.

Primer and Probes: The following information relates to the design and setup of TaqMan probe-based PCR. When using other probe types, please refer to the appropriate literature.

The sequence, concentration ratio of the primers to probes and the amplicon length, can be critical for specific amplification, yield and overall efficiency of any qPCR. We strongly recommend taking the following points into consideration when designing and running your qPCR:

- Use primer-design software, such as Primer3 (<http://frodo.wi.mit.edu/primer3/>) or visual OMPTM (<http://dnasoftware.com/>). Primers should have a melting temperature (T_m) of approximately 60°C; the T_m of the probe should be approximately 10°C higher than that of the primers.
- Optimal amplicon length should be 80-200 bp
- Final primer concentration of 400 nM is suitable for most reactions, however to determine optimal concentration we suggest titrating in the range of 0.2-1 µM.
- Final concentrations of 100 nM for each probe are adequate for most reactions; we recommend the final probe concentrations are at least 2-fold lower than the primer concentration.
- Use an equimolar primer concentration and an equimolar probe concentration

MgCl₂: The Liquid Biopsy Stool DNA qPCR Mix contains an optimized concentration of MgCl₂, it is not necessary to supplement the mix further.

PCR controls: It is important to detect the presence of contaminating DNA that may affect the reliability of the data. Always include a no-template control (NTC) reaction, replacing the template with PCR-grade water.

Procedure

Reaction mix composition: Prepare a PCR master mix. The volumes given below are based on a standard 20 µL final reaction mix and can be scaled accordingly.

Reagent	Volume	Final Concentration
Liquid Biopsy Stool DNA qPCR Mix, 4x	5 µL	1x
10 µM Forward Primer	0.8 µL	400 nM
10 µM Reverse Primer	0.8 µL	400 nM
10 µM Probe	0.2 µL	100 nM
Template	x µL*	
Total Volume	Up to 20 µL	

* The amount of inhibition tolerated by Liquid Biopsy Stool DNA qPCR Mix is variable depending on several factors, including assay design and sample quality. For this reason, an initial sample titration is recommended.

Suggested RT-qPCR conditions: The following RT-qPCR conditions are suitable for the Liquid Biopsy Stool DNA qPCR Mix with amplicons of up to 200 bp. However, the cycling conditions can be varied to suit different machine-specific protocols. It is not recommended to use annealing temperatures below 60°C.

Step	Temperature	Time	Cycles
Polymerase activation	95 °C	3 min	1
Denaturation	95 °C	10 s	45
Annealing/Extension**	60 °C	25 s	

** Annealing/Extension temperature needs to be optimised based on probes used.

Troubleshooting guide

Problem	Possible Cause	Recommendation
No amplification trace AND No product on agarose gel	Activation time too short	Ensure Liquid Biopsy Stool DNA qPCR Mix is activated for a minimum of 3 min at 95°C before cycling. For more complex templates, increase activation time up to 5 minutes.
	Error in protocol setup	Verify that correct reagent concentrations, volumes, dilutions and storage conditions have been used.
	Suboptimal primer design	Use primer design software or validated primers/probe. Test primers on a control Template.
	Incorrect concentration of primers/probe	Use primer concentration between 200 nM and 1 µM and probe concentration of 100 nM.
	Template degraded	Re-isolate your template from sample material or use freshly prepared dilutions.
	Primers/probe degraded	Use newly synthesized primers and probe.
	Template contaminated with PCR inhibitors	Further dilute template before qPCR or purify template and resuspend it in PCR-grade water.
	Template concentration too low	Increase concentration used.
Non-specific amplification product AND Primer-dimers	Cycling conditions not optimal	Increase extension/annealing times, increase cycle number.
	Suboptimal primer/probe design	Use primer/probe design software or validated assays. Test primer/probe on a control template.
	Primer/probe concentration too high	Test dilution series of primer concentrations until primer dimer/non-specific amplification products disappear.
	Primer/probe concentration too low	Use primer concentration between 200 nM and 1 µM and probe concentration of 100 nM.
	Primer annealing/extension temperature(s) too low	Due to the high ionic strength of Liquid Biopsy Stool DNA qPCR Mix it is not recommended to use annealing/extension temperatures below 60 °C. Annealing/extension temperature can be increased in steps of 2°C in the event of non-specific products.
	Template concentration too low	Increase template concentration.
	Template concentration too high	Reduce template concentration until non-specific products disappear.
Late amplification trace	Extension time too long	Reduce extension time to determine whether non-specific products are reduced.
	Activation time too short	Ensure the reaction is activated for between 3 min and 5min at 95°C before cycling.
	Extension time too short	Increasing the extension time may be necessary for amplification products over 200 bp; double extension time to determine whether the cycle threshold (Ct) is affected.
	Annealing temperature too high	Decrease annealing temperature in steps of 2°C.
	Template concentration too low	Increase concentration if possible.
	Template is degraded	Re-isolate template from sample material or use freshly prepared template dilution.
	Suboptimal design of primers	Redesign primers using appropriate software or use validated primers.
Primer/probe concentration too low	Use primer concentration between 200 nM and 1 µM and probe concentration of 100 nM.	

Associated Products

Product	Cat. No.
Genotyping Blood qPCR Mix	ODX001
Liquid Biopsy Blood DNA qPCR Mix	ODX002
Liquid Biopsy Blood RNA/DNA qPCR Mix	ODX003
Liquid Biopsy Urine DNA qPCR Mix	ODX004

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: +1 901 382 0027

Website: www.bioline.com email: info@meridianlifescience.com