# Liquid Biopsy Blood RNA/DNA qPCR Mix

## Product Handling Guide

| | |
|---|---|
| Shipping: | Blue Ice |
| Catalog number: | ODX003 |
| Batch No.: | See vial |
| Concentration: | 4x |

**Store at –20 °C**

**Storage and stability:**
Liquid Biopsy Blood RNA/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. Genotyping Blood 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 Blood RNA/DNA qPCR Mix is a one tube formulation combining the latest advances in buffer chemistry and PCR enhancers, together with an optimized concentration of antibody-mediated hot-start polymerase, reverse transcriptase, RNase Inhibitor, dNTPs and $MgCl_2$. Liquid Biopsy Blood RNA/DNA qPCR Mix has been designed for highly reproducible, accurate RNA and DNA target amplification, delivering excellent results in multiplex assays, even in the presence of whole blood and with different anticoagulants.

## Kit components

| Component |
|---|
| Liquid Biopsy Blood RNA/DNA qPCR Mix |

## General considerations

When handling RNA, it is important to use RNase-free plasticware and reagents. We also recommend performing RNA work in an RNase-free area. 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 DNA product are not opened in the reaction set-up area.

**Primer and Probes:** These guidelines refer to the use of dual-labeled probes. Please refer to the relevant literature when using other probe types. The sequence and concentration of the probe and primers, as well as amplicon length, can be critical for specific amplification, yield and overall efficiency of any RT-qPCR.

We strongly recommend taking the following points into consideration when designing and running your RT-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 (Tm) of approximately 60°C; the Tm 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

**$MgCl_2$:** The Liquid Biopsy Blood RNA/DNA qPCR Mix contains an optimized concentration of $MgCl_2$, 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 Blood RNA/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** | |

** Up to 10% whole blood or 20% serum or plasma can be used, however the amount of inhibition tolerated by Liquid Biopsy Blood RNA/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 Blood RNA/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 |
|---|---|---|---|
| Reverse transcription** | 50 °C | 10 min | 1 |
| Polymerase activation | 95 °C | 3 min | 1 |
| Denaturation | 95 °C | 15 s | 45 |
| Annealing/Extension** | 60-65 °C | 30 s | |

*** *When multiplexing, the reverse transcription reaction time can be extended up to 20 minutes and the annealing/extension time can be extended up to 60 seconds and/or the annealing/extension temperature can be increased up to 65° C.*

## Troubleshooting guide

| Problem | Possible Cause | Recommendation |
|---|---|---|
| No amplification trace AND No product on agarose gel | Activation time too short | Ensure Liquid Biopsy Blood RNA/DNA qPCR Mix is activated for a minimum of 3 min at 95°C before cycling. |
| | 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 assay 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 RT-qPCR or purify template and resuspend it in PCR-grade water. |
| | Template concentration too low | Increase concentration used. |
| | Cycling conditions not optimal | Increase extension/annealing times, increase cycle number. |
| Non-specific amplification product AND Primer-dimers | Inefficient reverse transcription | Extend reverse transcription time up to 20 min and/or increase the temperature up to 55 °C |
| | 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 Blood RNA/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. |
| | Extension time too long | Reduce extension time to determine whether non-specific products are reduced. |
| Late amplification trace | 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. |
| PCR efficiency below 90% | Extension time too short | Increase extension time. |
| | Primer concentration too low | Increase concentration of primers in 100 nM increments. |
| | Suboptimal primer/probe design | Redesign primer/probe using appropriate software or use validated primer/probe. |
| PCR efficiency Above 110% | Template is degraded or contains PCR inhibitors | Re-isolate template from sample material, or use freshly prepared template dilution, or purify template and resuspend in water. |
| | Non specific amplification and/or primer-dimers | Use 4% agarose gel electrophoresis to confirm presence of non-specific amplification products. See above for preventing/removing non-specific products |

### Associated Products

| Product | Cat. No. |
|---|---|
| Genotyping Blood qPCR Mix | ODX001 |
| Liquid Biopsy Blood DNA qPCR Mix | ODX002 |
| Liquid Biopsy Urine DNA qPCR Mix | ODX004 |
| Liquid Biopsy Stool DNA qPCR Mix | ODX005 |

### Technical Support

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

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