



Instructions for Use

LymphoTrack[®] Low Positive Controls & LymphoQuant[®] Internal Controls

Controls to enable streamlined monitoring of clonal cell equivalents and percent residual clonotype over time.

RUO These controls are for Research Use Only. Not for use in diagnostic procedures.

Manufactured in U.S.A.





Catalog

 REF
 40880098

 REF
 40880118

 REF
 40880108

 REF
 40880128

Products

LymphoTrack B-cell Low Positive Controls LymphoQuant B-cell Internal DNA Controls LymphoTrack T-cell Low Positive Controls LymphoQuant T-cell Internal DNA Controls

Storage Conditions: -30°C to -15°C

Quantity

1 Tube - 5 reactions 1 Tube - 120 reactions 1 Tube - 5 reactions 1 Tube - 120 reactions

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1. Product Use

The LymphoTrack Low Positive Control (RUO) and LymphoQuant Internal Control (RUO) are used in conjunction with LymphoTrack assays to estimate the rearranged cell equivalents within each sample tested. The LymphoTrack B-cell Low Positive Control and LymphoQuant B-cell Internal Controls can be used with the LymphoTrack *IGHV* Leader, *IGH* FR1, *IGH* FR2 and *IGH* FR3 Assays. The LymphoTrack T-cell Low Positive Control and LymphoQuant T-cell Internal Control can be used with the LymphoTrack *TRG* and *TRB* Assays. Refer to Figure 1 for suggested use of the LymphoTrack Low Positive and LymphoQuant Internal Controls.



Process the entire run with the appropriate LymphoTrack Assay and corresponding LymphoTrack Software.

Figure 1. The LymphoTrack MRD sample testing requires addition of the appropriate LymphoQuant Internal Control to each sample PCR replicate as well as the corresponding LymphoTrack Low Positive Control (only 1 PCR replicate). Please note that the LymphoQuant Internal control is not added to the PCR containing the Negative Control.

2. Summary

Minimal Residual Disease (MRD) testing by Next-Generation Sequencing (NGS) is a proven tool in the development of management strategies for hematologic malignancies. The LymphoTrack (RUO) Assays can be used for Minimal Residual Disease (MRD) assessment and tracking of rearranged clonal sequences.

- LymphoQuant B-cell or T-cell Internal DNA Controls are used to estimate cell equivalents for clonal populations provided sufficient DNA input is interrogated.
- LymphoTrack B-cell and T-cell Low Positive Controls are used to verify the sensitivity of the assay.
- The LymphoTrack MRD software enables streamlined monitoring of estimated clonal cell equivalents and percent residual clonotype over time.

Please refer to Table 1 as a guide for which controls to use for each LymphoTrack target.

Table 1.	Guide for LymphoTrack MRD	Controls for use with LymphoTrack MiSeq and Ion S5/PGM Assays
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LymphoTrack Target	LymphoTrack Low Positive Control	LymphoQuant Internal Control
IGHV Leader, IGH FR1/2/3	LymphoTrack B-cell Low Positive Control REF 40880098	LymphoQuant B-cell Internal Control REF 40880118
TRG, TRB	LymphoTrack T-cell Low Positive Control	LymphoQuant T-cell Internal Control

Note: IGHV Leader is only available for testing on the MiSeq instrument.

3. Precautions and Considerations

Sensitive, standardized testing such as NGS-based MRD may identify molecular relapse prior to other markers of disease presentation. The current gold standard MRD method, multiparametric flow cytometry, is highly subjective and recommendations are often based on consensus expert-shared knowledge and experience, not on a validated, objective method. The LymphoTrack suite of NGS assays offer a sensitive alternative and when used with the respective Low Positive Control (LPC) and LymphoQuant Internal Control (LQIC) controls offer a standardized workflow for MRD clonotype tracking. Precautions need to be taken to determine suitability of a clonal sequence for MRD tracking which are unique per locus and are outlined in the subsequent sections.

Note:	When selecting sequences for NGS-based MRD tracking, it is important to first verify that these clonal sequences are not detected in the negative control.
	 This negative control cannot be from the same flow cell as the clonal sequence source. Never run highly clonal samples on the same chip or flow cell as MRD samples.

3.1. Immunoglobulin heavy chain (IGH)

Due to the high genetic diversity of *IGH*, there are typically no considerations to be taken into account when selecting sequences for MRD tracking.

3.2. Immunoglobulin kappa chain (IGK)

The use of an *IGK* target for MRD testing is not ideal, and caution is warranted due to the low genetic diversity of this locus. The LymphoTrack B-cell Low Positive Control and LymphoQuant B-cell Internal Control cannot be used to with LymphoTrack *IGK* Assays. If alternate *IGK* controls are utilized, verify that any clonal *IGK* sequences identified are absent in negative control data before using the sequence for MRD tracking. There are many common rearrangement sequences that may be unsuitable for MRD analysis due to the high frequency in which they are observed in polyclonal negative samples. For example, any of the three clonotype sequences listed below may not be suitable for MRD analyses:

- Intron-Kdel
- V3D-20 with any J or Kdel
- V3-11 with any J or Kdel

3.3. T-cell receptor gamma (*TRG*)

The *TRG* locus is composed of fewer gene segments than *IGH*, *IGK*, and *TRB*, and thus is far less diverse. Due to this lack of diversity, *TRG* gene rearrangements of the same sequence are found between cells at a higher prevalence, increasing the likelihood of false positive MRD results. To minimize this risk, it is suggested to follow the following precautionary measures:

- 3.3.1. Track only **exact match sequences** to reduce the likelihood of a false positive match. In other loci, tracking up to two mismatches is useful for accounting for sequencing error; however, using this method for the *TRG* locus will exacerbate the false positive frequency.
- 3.3.2. Track **two clonal sequences** to reduce the probability of a false positive match. The *TRG* locus has a strong propensity for bi-allelic rearrangements and will display as two clonal sequences in the LymphoTrack output. Use both of these sequences for MRD tracking. In cases of a bi-clonal sample, the likely output will include more than two clonal sequences and provide multiple trackable sequences that can improve the specificity of the assay.

3.4. T-cell receptor beta (*TRB*)

When tracking *TRB* gene rearrangements, please note that D-J rearrangements are less suitable for MRD testing due to the reduced diversity found in these immature rearrangements. To minimize the risk of false positive MRD results, it is also suggested to use exact sequence matches for *TRB*-based MRD tracking.

4. Controls

4.1. LymphoTrack Low Positive Controls

The LymphoTrack Low Positive Controls are DNA extracted from known gene rearrangement cell lines grown under standard cell culture conditions. These Research Use Only (RUO) DNA controls are prepared in a polyclonal background and can be used as the run validity controls for each LymphoTrack sample when testing samples at low sensitivity. Please refer to Table 2 for available LymphoTrack Low Positive Controls.

Table 2. LymphoTrack Low Positive Controls

Reagent/Material	Suppliers	Catalog #	Notes
LymphoTrack B-cell Low Positive Control*	Invivoscribe, Inc.	REF 40880098	Average expected read frequency is 10 ⁻⁴
LymphoTrack T-cell Low Positive Control [§]	Invivoscribe, Inc.	REF 40880108	Average expected read frequency is 10^{-4}
LymphoTrack MRD Software	Invivoscribe, Inc.	REF 75000008	Version 1.2.0 or later

Note: The LymphoTrack B-cell Low Positive Control can be used with the LymphoTrack *IGHV* Leader and *IGH* FR1/FR2/FR3 assays.
 Note: The LymphoTrack T-cell Low Positive Control can be used with the LymphoTrack *TRG* and *TRB* assays.
 Note: The *TRB* loci generate a read frequency of 10⁻³.

4.2. LymphoQuant Internal Controls

The LymphoQuant Internal Controls are DNA extracted from known gene rearrangement cell lines grown under standard cell culture conditions. These Research Use Only (RUO) DNA controls are used as the internal controls spiked at known cell equivalents into each sample in conjunction with LymphoTrack assays (Table 1).

Use of a LymphoQuant Internal Control with the LymphoTrack MRD software allows the number of clonotype cell equivalents and frequency in the sample to be calculated and reported, enabling accurate monitoring of hematologic disease in longitudinal studies. Please refer to Table 3 for available LymphoQuant Internal DNA Controls, which can facilitate standardized MRD assessment and monitoring.

Table 3. LymphoQuant Internal DNA Controls

Reagent/Material	Suppliers	Catalog #	Notes
LymphoQuant B-cell Internal Control [‡]	Invivoscribe Inc.	REF 40880118	50 cell equivalents/μL
LymphoQuant T-cell Internal Control [§]	Invivoscribe Inc.	REF 40880128	50 cell equivalents/µL
LymphoTrack MRD Software	Invivoscribe Inc.	REF 75000008	Version 1.2.0 or later

***Note:** The LymphoQuant B-cell Internal Control can be used with the LymphoTrack *IGHV* Leader and *IGH* FR1/FR2/FR3 assays.

Note: IGHV Leader is only available for testing on the MiSeq instrument.

[§]Note: The LymphoQuant T-cell Internal Control can be used with the LymphoTrack TRB and TRG assays.

5. Minimal Residual Disease (MRD) Application

This protocol is to be used in conjunction with and as an addendum to the respective LymphoTrack Assay Instructions for Use (IFU) for the target being tested.

 Note:
 In any high-throughput sequencing technology that utilizes sample barcodes such as the MiSeq and Ion S5/PGM, the high number of clonotype sequences present in a highly clonal sample may contaminate subsequent samples if precautions are not taken. To mitigate the risk of sequencing artifacts, Invivoscribe provides the following guidelines:

 For MiSeq:

 Conduct an Illumina *Template Line Wash* with bleach after each MiSeq run.
 Avoid running subsequent time points immediately after a MiSeq run that contained the highly clonal sample.

 For MiSeq or Ion S5/PGM:

 Test follow up samples separately from the highly clonal sample.
 Use different indices for the highly clonal sample and follow-up samples (*e.g.,* index 1 for identifying the clonotype sequence and index 2 for a follow up sample from the same subject). Alternatively, unrelated samples may be run between the highly clonal sample and follow-up runs on the same instrument as long as a template line wash with bleach is conducted.

• Avoid running known highly clonal samples with follow-up samples screened at high-read depths on the same chip or flow cell.

5.1. DNA Input Quantity

DNA input amount is a critical factor of experimental design. Higher DNA input is suggested when performing MRD testing because the overall cell equivalents surveyed determines the sensitivity of an MRD assay. When using LymphoTrack assays, a maximum DNA input of 2 µg per PCR replicate is recommended. Please refer to Table 4 below for the suggested sample testing scheme (i.e. number of PCR replicates per sample) to achieve desired sensitivity.

NOTE: The level of confidence for detecting a clonotype (detected with at least 5 reads) at various DNA input quantities and replicates is a function of the number of sequencing reads obtained. The confidence levels for detecting clonotype sequences depicted in Table 4 were calculated using a statistical model. This model does not incorporate PCR bias; therefore, the calculated confidence levels are theoretical and not empirically determined.

95% Confidence of a True MRD Negative Sample at Various Sensitivity Levels					
Sensitivity	DNA per PCR replicate	# of PCR replicates	Read depth per replicate		
1 × 10 ⁻³	20 ng	1 replicate of 20 ng	50,000		
1 × 10 ⁻⁴	200 ng	1 replicate of 200 ng	500,000		
1 10-5	700 ng	5 replicates of 700 ng each	700,000		
1 × 10 ⁻³	2 µg	2 replicates of 2 μ g each	1,400,000		

Table 4. Examples of DNA input and corresponding read depth.

Note: The number of indices sequenced per flowcell/chip affects read depth; the number of samples per run may need to be adjusted to achieve the desired read depth per replicate.

5.2. Reagent Preparation (continued from step 7.3.1 of the LymphoTrack Assay protocol)

- 5.2.1. In a containment hood or dead air box, pipette 45 μL from each Master Mix tube into a clean PCR plate (one well for each Master Mix and one Master Mix per replicate).
 - Include two DNA controls in each run (one LymphoTrack Low Positive Control and one negative control) as well as one NTC (using molecular biology grade water as template instead of DNA).
 - Run each Sample and Low Positive Control with the (spike-in) LymphoQuant Internal Control.
- 5.2.2. Add 0.2 µL of Taq DNA Polymerase (@5 U/µL) to each well containing aliquoted Master Mixes.
- 5.2.3. Add the appropriate template to the individual wells containing the respective Master Mix reactions, following the guidelines below (summarized in Table 5):
 - 10 μL of molecular biology grade water (NTC) or Negative Control
 - 8 μL of LymphoTrack Low Positive Control
 - Up to 8 μL of sample DNA (QS with molecular grade water)
- 5.2.4. Add 2 µL of LymphoQuant Internal Control to each of the individual wells containing PCRs with testing samples and the positive control.
 - Pipette up and down 5-10 times to mix using a pipette set to 55 μL.
 - Seal the plate, briefly centrifuge and place in the PCR thermal cycler.

Table 5. PCR Setup

Component	Low Positive Control	MRD Sample [§] with LymphoQuant Control	Negative Control REF 40920018	No Template Control
Master Mix	45.0 μL	45.0 μL	45.0 μL	45.0 μL
Taq DNA polymerase	0.2 μL	0.2 μL	0.2 μL	0.2 μL
Template	8.0 μL	8.0 μL	10.0 μL	10.0 μL
LymphoQuant Internal Control	2.0 μL	2.0 μL	-	-
Total	55.2 μL	55.2 μL	55.2 μL	55.2 μL

[§]Note: If the MRD Sample DNA input volume is less than 8.0 μL, QS with molecular grade water.

- 5.2.5. Refer to step 7.4.1 of the LymphoTrack Assay IFU for amplification and thermal cycling conditions.
 - After amplification completes, store the PCR products at 4°C for up to one day or continue to step 7.5 of the LymphoTrack Assay IFU.
- 5.2.6. Follow the steps listed under section 7.5 of the respective LymphoTrack Assay IFU, modifying the AMPure XP reagent volumes to maintain the correct ratio of magnetic particles to PCR volume (follow instructions in Table 6):

LymphoTrack MRD target(s)	Sequencing Platform	PCR volume	volume ratio (PCR : AMPure XP reagent)	AMPure XP reagent volume
IGHVI eader IGH	MiSeq	55.2 μL	1:1	55.0 μL
FR1/FR2/FR3	lon S5/PGM	55.2 μL	1:1.8	99.0 μL
TRC	MiSeq	55.2 μL	1:1	55.0 μL
780	lon S5/PGM	55.2 μL	1:1.8	99.0 μL
TRB	MiSeq	55.2 μL	1:0.7	38.5 μL

Table 6. AMPure XP reagent volumes

5.2.7. After completing the AMPure XP reagent purification and elution, refer to the LymphoTrack Assay IFU starting at section 7.6 for quantification and continue following the IFU through template preparation and sequencing steps.

NOTE: Use the read length/flows listed in the respective LymphoTrack Assay IFU.

5.3. Sequencing Reagents

Reagent / Material	Recommended Reagents / Suppliers	Catalog #
lon S5 Sequencing	Thermo Fisher Scientific: • Ion 510 & Ion 520 & Ion 530 Kit – Chef	REF A34019
MiSeq Sequencing	Illumina: • MiSeq Reagent v3 kit (600 cycles)	REF MS-102-3003

 Table 7.
 Recommended Sequencing Reagent kits for MRD applications

6. Data Analysis

The LymphoTrack Low Positive Controls and LymphoQuant Internal DNA Controls were designed to produce sequencing data that can be analyzed using the LymphoTrack MRD Software, provided on the associated CD or downloaded from the Invivoscribe software portal (REF 7500008). Please see the LymphoTrack MRD Software IFU (https://catalog.invivoscribe.com/product/75000008/) for detailed instructions to install and use the software.

7. Technical and Customer Service

Thank you for purchasing our LymphoQuant and LymphoTrack MRD controls. We appreciate your business. We are happy to assist you with understanding this assay and will provide ongoing technical assistance Monday through Friday to keep the assays performing efficiently in your laboratory.

Contact Information

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Invivoscribe, Inc
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 10222 Barnes Canyon Road
 | Building 1 | San Diego | California 92121-2711 | USA

 Phone: +1 858 224-6600 | Fax: +1 858 224-6601 | Business Hours: 7:00AM - 5:00 PM PST/PDT

 Technical Service: support@invivoscribe.com | Customer Service: sales@invivoscribe.com | Website: www.invivoscribe.com

8. References

- LymphoTrack MRD Software (REF 75000008)
- LymphoTrack Software S5/PGM (REF 75000007)
- LymphoTrack Software MiSeq (REF 75000009)
- LymphoTrack IGH FR1 Assay Kit A MiSeq (REF 71210009, document 280377)
- LymphoTrack IGH FR1 Assay Panel MiSeq (REF 71220007, document 280377)
- LymphoTrack IGH FR1 Assay Panel B MiSeq (REF) 71220007, document 280377)
- LymphoTrack IGH FR2 Assay Kit A MiSeq (REF 71220007, document 280377)
- LymphoTrack *IGH* FR2 Assay Panel MiSeq (REF 71220007, document 280377)
- LymphoTrack *IGH* FR3 Assay Kit A MiSeq (REF 71220007, document 280377)
- LymphoTrack IGH FR3 Assay Panel MiSeq (REF 71220007, document 280377)
- LymphoTrack IGH FR1/2/3 Assay Kit A MiSeq (REF 71220007, document 280377)
- LymphoTrack IGH FR1/2/3 Assay Panel MiSeq (REF 71220007, document 280377)
- LymphoTrack IGHV Leader Somatic Hypermutation Assay Kit A MiSeq (EEE 71210059, document 280337)
- LymphoTrack IGHV Leader Somatic Hypermutation Assay Panel MiSeq (EEE 71210069, document 280337)
- LymphoTrack TRB Assay Kit A MiSeq (REF 72250009, document 280412)
- LymphoTrack TRB Assay Panel MiSeq (REF 72250019, document 280412)
- LymphoTrack TRG Assay Kit A MiSeq (REF 71220009, document 280323)
- LymphoTrack TRG Assay Panel MiSeq (REF 71220019, document 280323)
- LymphoTrack *IGH* FR1 Assay S5/PGM (EEF 71210007, document 280376)
- LymphoTrack IGH FR2 Assay S5/PGM (REF 71210037, document 280376)
- LymphoTrack *IGH* FR3 Assay S5/PGM (REF 71210047, document 280376)
- LymphoTrack IGH FR1/2/3 Assay S5/PGM (REF 71210057, document 280376)
- LymphoTrack TRG Assay S5/PGM (REF 72270007, document 280354)

9. Symbols

The following symbols are used in labeling for Invivoscribe NGS products.



10. Legal Notice

10.1. Warranty and Liability

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