Invivoscribe Products Training

Controls, Reagents and Enzymes

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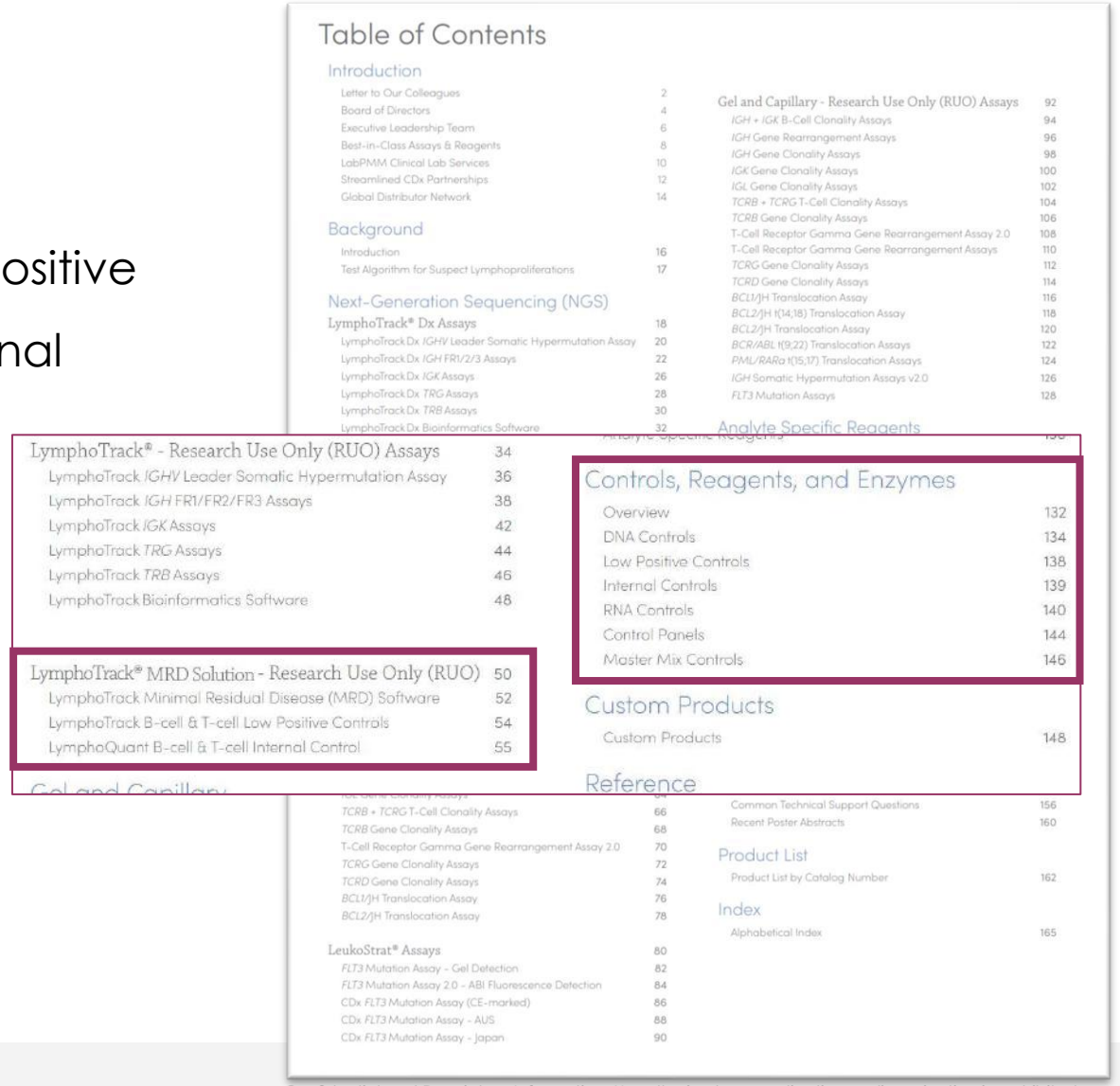
Invivoscribe Controls Portfolio

Controls, Reagents and Enzymes

IVS Portfolio:

- LymphoTrack® RUO NGS Controls:
  - LymphoTrack® B-Cell and T-Cell Low Positive
  - LymphoQuant® B-Cell and T-Cell Internal

- Controls Reagents and Enzymes:
  - DNA Controls
  - Low Positive Controls
  - Internal Controls
  - RNA Controls
  - Master Mix Controls
  - ABI Detection Reagents
  - EagleTaq DNA Polymerase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Controls

IVS Control Usage

- DNA & RNA Controls Represent a Huge Market
  - Laboratories need positive and negative controls for all assays
  - Controls are needed for sensitivity, proficiency & troubleshooting
- Suitable Quality Controls are Difficult to Obtain
  - Laboratories struggle to obtain all necessary controls
  - Require a qualified source & manufacturing capabilities
- Controls are often Overlooked Revenue Opportunities
  - Represent 33% of US market for IVS

Controls

IVS is Known for Offering High Quality Controls

- Laboratories appreciate that IVS kits include all necessary controls
- Additional controls are available that can be used to complete our suite of assays
- Individual DNA & RNA controls are available containing specific mutations, translocations, clonal rearrangements at various dilutions
- Controls manufactured under **cGMP*** conditions

*GPRs and CE-marked controls are manufactured under cGMP.

Controls and Reagents

Quick Reference for DNA Controls

The vast majority of our high-quality DNA controls, including sensitivity controls and panels, are supplied in aliquots of 100 µL and are adjusted to a final concentration of 200 µg/mL in 1/10 TE (1 mM Tris- HCl (pH 8.0), 0.1 mM EDTA).

Positive for	Immunoglobulin Rearrangements			Mutations			Translocations		T-Cell Receptor Gene Rearrangements		
	IGH	*IGK*	*IGL*	*IGHV* SHM	*FLT3* ITD	*FLT3* TKD	*IGH-CCND1***	*IGH-BCL2*	*TRB*	*TRG*	*TRD*
IVS-0001								◆			
IVS-0004									◆	◆	
IVS-0007	◆	◆	◆					◆			
IVS-0008[€]	❍								◆	◆	
IVS-0009									◆	◆	
IVS-0010	◆	◆					◆				
IVS-0013	◆	◆									
IVS-0019	◆	◆									
IVS-0021		◆							◆	◆	◆
IVS-0024	◆	◆									
IVS-0029	◆	◆									
IVS-0030 [‡]	◆	◆		◆							
IVS-0031	◆	◆									
IVS-P002											
LymphoQuant B-cell Internal Control	★	★									
LymphoQuant T-cell Internal Control									★	★	
LymphoTrack B-cell Low Positive Control	★	★									
LymphoTrack T-cell Low Positive Control									★	★	
FLT3 ITD Positive Control					◆						
FLT3 TKD Positive Control						◆					

◆ Gene rearrangement ❍ Partial *IGH* DH-JH rearrangement ★ Recommended for NGS

[€]This control does not contain a complete *IGH* VH-JH rearrangement and may only be suitable for *IGH* DH-JH rearrangements.

**IGH-CCND1* was previously referred to as *BCL1/JH*

[‡]These controls can be used as SHM positive controls with ≥2% mutational rates compared to the germline sequence.

LymphoTrack® MRD Controls

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LymphoTrack® MRD Products

Assay Kits and Controls overview:

Catalog #	Description	Components
Various	LymphoTrack® (Dx) Assay Kit	*IGHV* Leader, *IGH* FR1/FR2/FR3 or *IGK* master mixes and controls
4-088-0108	LymphoTrack® T-cell Low Positive Control	50 µL DNA Control, 5 reactions
4-088-0128	LymphoQuant® T-cell Internal Control	250 µL DNA Control, 120 reactions
4-088-0098	LymphoTrack® B-cell Low Positive Control	50 µL DNA Control, 5 reactions
4-088-0118	LymphoQuant® B-cell Internal Control	250 µL DNA Control, 120 reactions
7-500-0008	LymphoTrack® MRD Software	CD with Software and IFU

LymphoTrack® MRD Controls

LymphoTrack® Low Positive Control (LPC)

- Purpose
  - The LPC serves as the **MRD run validity control** and is used in place of the LymphoTrack® kit Positive Control. The LPC **confirms a run sensitivity threshold** is achieved.
- Description
  - This is a low level DNA control extracted from a known gene rearrangement cell line dilution for use with LymphoTrack T- or B-cell assays for MRD testing.
  - Average expected read frequency is **10^{-4} *.**
  - 1 tube (50 µL). Sufficient for 5 LPC reactions at 8 µL each.

- **Research Use Only**

*The *IGK* and *TRB* loci generate a read frequency of 10^{-3}

LymphoTrack® MRD Controls

LymphoQuant® Internal Control (LQIC)

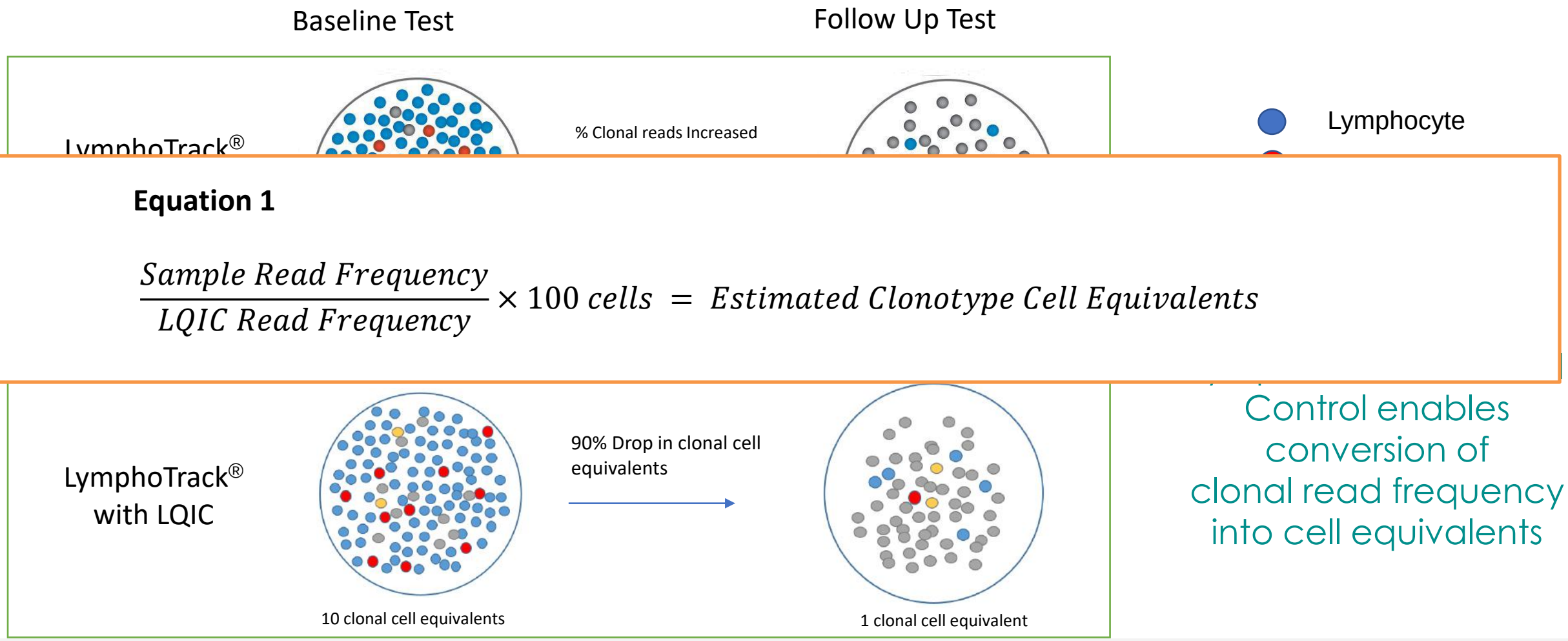
- Purpose
  - The LQIC was created to aid in objective MRD tracking by providing an **estimate of clonal burden or clonal cell equivalents**.
- Description
  - This control is DNA extracted from a known gene rearrangement cell line that is diluted to 50 cell equivalents per µL.

    2 µL generate read numbers equivalent to 100 cells.
  - 1 tube (250 µL). 2 µL per reaction or LQIC spike-in.

- **Research Use Only**

LymphoTrack® MRD Controls

Principle Behind Internal Calibration:



Equation 1

$$\frac{Sample\ Read\ Frequency}{LQIC\ Read\ Frequency} \times 100\ cells = Estimated\ Clonotype\ Cell\ Equivalents$$

Control enables conversion of clonal read frequency into cell equivalents

LymphoTrack® MRD Controls

LymphoQuant® Internal Control (LQIC)

- Software automatically calculates the estimated Clonal Cell Equivalents indicated in the report:

Minimal Residual Disease (MRD) Summary Table

LymphoTrack MRD Report 2.0.1_ALPHA

Subject ID	Test-Subject 1	Gene Target	IGH FR1
Sequence Name	Sequence_1	Sequence #	1

NOTE: Use of the same sample type is recommended to most accurately track clonal cell equivalents over time.

Collection Date	Sample Unique Identifier	Sample Type	Estimated Clonal Cell Equivalents	Clonal Frequency
2018/09/01	Initial Sample	Bone Marrow	1094.81	1.43E-1
2018/12/01	Follow-up Sample 1	Bone Marrow	NOT DETECTED	NOT DETECTED
2019/03/01	Follow-up Sample 2	Bone Marrow	NOT DETECTED	NOT DETECTED
2019/06/01	Follow-up Sample 3	Bone Marrow	9.67	3.15E-5

*The *IGK* and *TRB* loci generate a read frequency of 10^{-3}

Controls:

Master Mix-, DNA-, RNA- and Panel Controls

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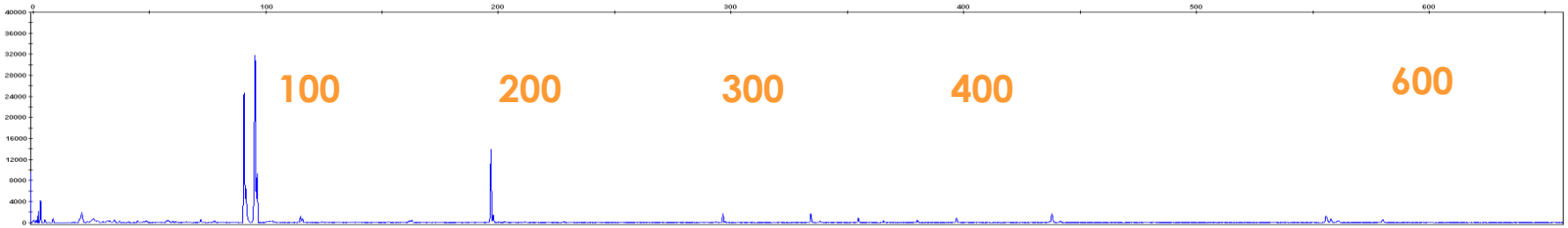
Master Mix Controls

Specimen Control Size Ladder

- Primers Designed by BIOMED-2 Group
- Targets 4 Housekeeping Genes
- **100, 200, 300, 400** and **600 bp** Products
- Can be used for other assays other than clonality to test **sample quality**
- Ensure that the quality and quantity of the sample DNA is adequate to yield a valid result



Degraded DNA

DNA Controls

Our Positive DNA controls can be used for most assays targeting:

- B- and T-cell antigen receptor loci
- *FLT3* ITD and TKD loci
- *IGH-BCL2*, *BCR-ABL*, and *PML-RARa* translocations

The vast majority of our DNA controls

are supplied in 100 µL aliquots of 200 µg/mL

DNA Controls

Negative Polyclonal Control DNA

- **IVS-0000 Polyclonal Control DNA** is genomic DNA isolated from the tissue of normal human tonsils
- IVS-0000 DNA is an excellent negative control for
  - Gene rearrangements
  - Chromosome translocations
  - Mutations
  - Clonality
- Included in all of our DNA based assay kits

Control Panels

DNA Sensitivity Panels

- 6 member panels that consist of 100% clonal DNA extracted from a positive control cell line and
- 30%, 20%, 10%, 5% and 1% dilutions of the positive DNA diluted (V/V) into our standard
- Each tube contains 100 µL of DNA at 200 µg/mL in 1/10 TE
- **These controls are qualitative**

DNA Sensitivity Panels		
Catalog #	Description	Can be used as a positive control for:
4-086-0040	Sensitivity Panel for IVS-0004 Clonal Control DNA	*TRB, TRG*
4-086-0070	Sensitivity Panel for IVS-0007 Clonal Control DNA	*IGH, IGK, IGL, IGH-BCL2* t(14;18) Mbr
4-086-0090	Sensitivity Panel for IVS-0009 Clonal Control DNA	*TRB, TRG*
4-086-0100	Sensitivity Panel for IVS-0010 Clonal Control DNA	*IGH, IGK, IGL, IGH-CCND1* t(11;14)
4-086-0190	Sensitivity Panel for IVS-0019 Clonal Control DNA	*IGH, IGK*
4-086-0210	Sensitivity Panel for IVS-0021 Clonal Control DNA	*TRB, TRD, TRG*
4-086-0300	Sensitivity Panel for IVS-0030 Clonal Control DNA	*IGH, IGK, IGH-BCL2* t(14;18) Mbr

Control Panels

RNA Sensitivity Panels

- **7 member panels** that consist of 100% clonal RNA extracted from a positive control cell line and
- 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions of the positive RNA diluted (V/V) into our standard
- Each tube contains 100 µL of RNA at 400 µg/mL in RNase-free glass-distilled water
- **These controls are qualitative**

RNA Sensitivity Panels		
Catalog #	Description	Can be used as a positive control for:
4-087-0030	Sensitivity Panel for IVS-0003 Clonal Control RNA	*BCR-ABL1* t(9;22) p210 e13a2 (b2a2)
4-087-0110	Sensitivity Panel for IVS-0011 Clonal Control RNA	*BCR-ABL1* t(9;22) p210 e14a2 (b3a2)
4-087-0150	Sensitivity Panel for IVS-0015 Clonal Control RNA	*CBFB/MYH11* inv16
4-087-0200	Sensitivity Panel for IVS-0020 Clonal Control RNA	*PML-RARA* t(15;17) L-form
4-087-0320	Sensitivity Panel for IVS-0032 Clonal Control RNA	*BCR-ABL1* t(9;22) p190 e1a2

Control Panels

Proficiency Panel for *BCR-ABL1* t(9;22)

- Used to **develop tests that identify *BCR-ABL1* t(9;22) translocations** and designed to be used with the *BCR/ABL* t(9;22) Translocation Assay Kits.
- **10 member panel** consisting of 100% clonal control RNA extracted from three *BCR-ABL1* positive cell lines as well as
- 10^{-2} and 10^{-4} dilutions of these three cell lines into the negative IVS-0035 clonal control RNA
- A sample of 100% IVS-0035 clonal control RNA is also included

Ordering Information

Catalog #	Description
4-310-0100	Proficiency Panel for the *BCR/ABL* t(9;22) Translocation Assay

RNA Proficiency Panel

Qty	Description	Chromosome Translocation
1	IVS-0003 Clonal Control RNA 10^{-2} IVS-0003 Clonal Control RNA 10^{-4} IVS-0003 Clonal Control RNA	*BCR-ABL1* p210 e13a2 (b2a2)
1	IVS-0011 Clonal Control RNA 10^{-2} IVS-0011 Clonal Control RNA 10^{-4} IVS-0011 Clonal Control RNA	*BCR-ABL1* p210 e14a2 (b3a2)
1	IVS-0032 Clonal Control RNA 10^{-2} IVS-0032 Clonal Control RNA 10^{-4} IVS-0032 Clonal Control RNA	*BCR-ABL1* p190 e1a2
1	IVS-0035 Clonal Control RNA	*BCR-ABL1* Negative

Control Panels

BCR/ABL RNA Dilution Sets

- Can be used as a reference and for development of materials with assays that target specific transcripts
- 6 member panel containing RNA from *BCR-ABL1* positive cell lines at 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} dilutions and 100% *BCR-ABL1* negative RNA

Ordering Information – e14a2 (b3a2)

Catalog #	Description
4-085-0210	*BCR/ABL* b3a2 RNA Dilution Set (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} dilutions and negative)

Ordering Information – e13a2 (b2a2)

Catalog #	Description
4-085-0310	*BCR/ABL* b2a2 RNA Dilution Set (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} dilutions and negative)

Ordering Information – e1a2

Catalog #	Description
4-085-0110	*BCR/ABL* e1a2 RNA Dilution Set (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} dilutions and negative)

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Reagents and Enzymes

Reagents

ABI Detection Reagents

- Invivoscribe also offers **highly deionized (Hi-Di) Formamide with ROX size standards** for ABI fluorescence detection with the **ABI 310 or 3100** series.
- For samples tested on an **ABI 310 or 3100** series, we recommend using the Hi-Deionized Formamide with ROX Size Standards mixture for PCR products
- For samples tested on an **ABI 3500** series use **GeneScan™ 600® LIZ dye Size Standard v2.0**

Ordering Information	
Catalog #	Description
6-098-0051	Hi-Deionized Formamide with ROX Size Standard (ABI 310), 1 mL
6-098-0061	Hi-Deionized Formamide with ROX Size Standard (ABI 3100), 1 mL
Available through Thermo Fisher Scientific®: 4408399	GeneScan™ 600 LIZ® dye v2.0 Standard (ABI 3500), 800 reactions

Enzymes

EagleTaq™ DNA Polymerase

- EagleTaq DNA Polymerase can be used to obtain highly specific and sensitive PCR amplification products
- This enzyme has been proven to minimize extension of non-specifically bound primers
- Obtain reliable results by using the gold standard of hot start polymerases for robust performance

Ordering Information	
Catalog #	Description
6-097-0100	EagleTaq DNA Polymerase 1000 U, 5 U/µL

Note: This product is for sale and use in the European Economic Area only. It is not to be resold or transferred to another party

Take Home Messages

Invivoscribe offers:

- **High quality DNA & RNA controls** manufactured under **cGMP*** conditions
- Controls compatible with GEL, ABI and NGS assays
- The **first MRD controls** on the market
- The **only Internal NGS MRD Control** enabling the conversion of clonal read frequency into cell equivalents

Invivoscribe controls can be used for non Invivoscribe assays
(Multiple targets and LDTs)

*GPRs and CE-marked controls are manufactured under cGMP.

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Controls, Reagents and Enzymes

Quiz

Quiz

Who are potential customers for Invivoscribe controls?

1. Existing Invivoscribe assay users and customers
2. Homebrew users
3. Users of assays targeting B-cell and T-cell antigen receptor loci, *FLT3* ITD and TKD loci or *IGH-BCL2*, *BCR-ABL1* and *PML-RARa* chromosome translocations
4. All of the above

Quiz

For which ABI instruments does Invivoscribe offer reagents?

1. ABI 310
2. ABI 3500
3. ABI 3500 XL
4. ABI PRISM 3700
5. ABI PRISM 7500

Quiz

What does the IVS-0000 Polyclonal DNA control consist of?

1. Genomic DNA isolated from the tissue of normal human tonsils
2. RNA isolated from the tissue of normal human tonsils
3. Clonal Control DNA

Quiz

Which is true for the LymphoTrack® Low Positive Control (LPC)?

1. The LPC confirms a run sensitivity threshold is met
2. One tube is sufficient for 10 LPC reactions at 8 µL each

Quiz

Which data is needed to calculate the Clonotype Cell Equivalents with LymphoQuant?

- % total reads of the MRD analysis
- Sample Read Frequency
- LQIC Read Frequency
- % total reads of the Clonality analysis

Equation 1

$$\frac{Sample\ Read\ Frequency}{LQIC\ Read\ Frequency} \times 100\ cells\ =\ Estimated\ Clonotype\ Cell\ Equivalents$$