PhiRx™ Indexed Control

for Illumina® Sequencing Platforms

Catalog numbers:
300184: PhiRx Indexed Control

User Guide

v20251009

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Overview

PhiRx Indexed Control is a dual-indexed control library, made from phiX174 genomic DNA, optimized for Illumina sequencing platforms, particularly two-color systems like XLEAP SBS chemistry on NextSeq 1000/2000 and NovaSeq X. By improving color balancing for low-plexity libraries our PhiRx Indexed Control ensures cleaner, more accurate sequencing results.

Key Features

- **10 bp i5 and i7 indexes with mixed bases**: Provides high base diversity, automatically meeting color balancing requirements on supported platforms. (patent-pending)
- **Color balancing optimization**: Specifically designed for the sensitive XLEAP SBS chemistry, enabling as little as 5% spike-in to correct base calling issues in libraries with low-diversity indexes.
- **High diversity for low-plexity samples**: Randomized inserts with 45% GC content improve sequencing accuracy for low-diversity libraries.
- **Reliable quality control**: Derived from the well-characterized phiX174 genome, providing robust calibration to support sequencing accuracy and performance.

Specifications

- **Product Type:** Non-denatured, double-stranded PhiRx Indexed Control solution.
- **Volume and Concentration:** Provided as 10 µL of 10 nM library solution.
- **Buffer:** Supplied in 10 mM Tris-HCl, pH 8.0.
- **Storage Conditions:**
    - **Short-term storage:** Aliquots and dilutions should be stored at 4°C.
    - **Long-term storage:** Store at -20°C for prolonged stability. Avoid repeated freeze-thaw cycles to maintain library integrity.

- **Library Size Distribution:**

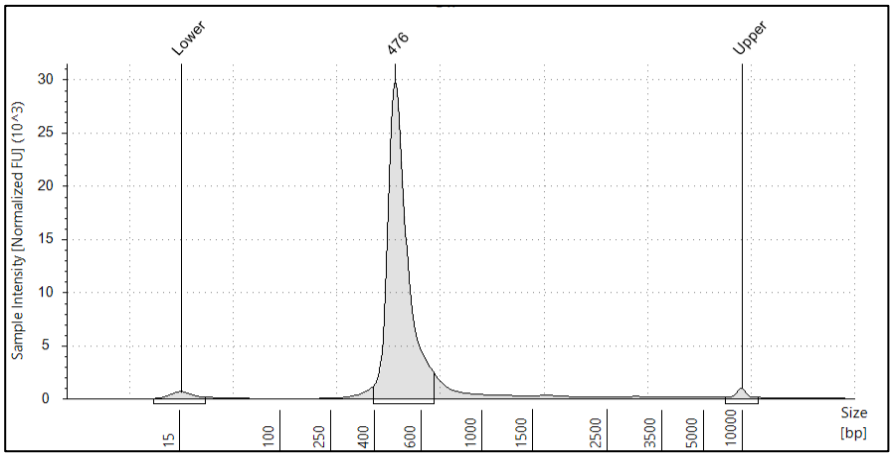

Figure 1. A representative electropherogram of the PhiRx Indexed Control size distribution

Product Components

PhiRx Indexed Control

Catalog No.: 300184

Item	Component	REF	Description	Storage	Qty
1	PhiRx Indexed Control	300184	10 µL of 10nM PhiRx Indexed Control solution in a screwcap tube	-25° to -15°C	1

User-Supplied Reagents, Equipment, and Consumables

Reagents:

Please refer to Illumina's sequencer-specific recommendations for dilution and loading for required reagents: https://knowledge.illumina.com/instrumentation/general.

Equipment & Consumables

- Single-channel pipettor
- Pipette tips (low-retention barrier tips)
- Benchtop centrifuge to pulse-spin tubes

Before starting procedure:

Review FAQs. FAQs can be found here: https://seqwell.com/resource-category/faqs/. Review these prior to your first use.

Thaw and mix reagent. Thaw reagent if necessary, pulse-spin down, and keep on ice. Pipette mix 10x before using.

Procedure

1. Dilution, aliquoting and denaturation

Please refer to Illumina's sequencer-specific recommendations for dilution and loading: https://knowledge.illumina.com/instrumentation/general.

PhiRx Indexed Control is a **non-denatured**, double-stranded library that is ready to be mixed with **non-denatured** target libraries. For applications requiring a denatured format, PhiRx Indexed Control can also be denatured following standard procedures and mixed exclusively with other **denatured** libraries.

Follow the procedure below for proper dilution, aliquoting, and storage:

a. **Non-Denatured Use**

   i. **Determine Concentration**: Select the appropriate concentration and aliquot volume based on your sequencing run's loading requirements.

   ii. **Low-Concentration Dilutions**: For dilutions below 1 nM, prepare aliquots sized for 1–2 weeks of use. Store long-term aliquots at -20°C, keeping only the aliquot in active use at 4°C.

b. **Denatured Use**

   i. **Dilution and Denaturation**: Dilute PhiRx Indexed Control to the required concentration, then denature according to Illumina’s standard protocol, mixing by vortex and briefly spinning down.

   ii. **Storage**: Store the denatured PhiRx Indexed Control at -20°C. Prepare single-use aliquots, discarding any leftover volume after loading to prevent freeze-thaw degradation.

2. Sequencing on Illumina platforms

Check Illumina recommendations specific to your sequencing platform and analysis software version to determine the appropriate PhiRx Indexed Control spike-in percentage.

Recommended Spike-In Ratios by Application:

a. **For Sequencing Quality Monitoring**: Add 1–2% of PhiRx Indexed Control to the target library.

b. **For Color Balancing of Index Reads with XLEAP-SBS**: Begin with 5–10% PhiRx Indexed Control. Per Illumina's recommendation for their PhiX Sequencing Control V3, using a higher percentage of PhiRx (up to 40%) may improve performance, especially with XLEAP-SBS 600-cycle kits.

c. **For Low-Diversity Libraries (Color Balancing of Insert Reads)**: Start with 15–20% PhiRx Indexed Control and adjust upward if needed to enhance sequencing performance.

3. Sample sheet setup and bioinformatics information

PhiRx Indexed Control is designed with randomized mixed bases at each index position, meaning it cannot be demultiplexed from the run as a distinct sample. As with PhiX Sequencing control V3, PhiRx reads will appear in the undetermined category after demultiplexing, so no additional information is required during sample sheet setup.

For additional support with read filtering or bioinformatics applications, please contact technical support at support@seqwell.com.

Technical Assistance

Please review FAQs at https://seqwell.com/resource-category/faqs/.
For additional technical assistance with PhiRx Indexed Control, contact seqWell Technical Support.

E-mail: support@seqwell.com

Version	Release Date	Prior Version	Description of changes
V20251009	**October 09, 2025**	**N/A**	**First Version**

seqWell, Inc.
66 Cherry Hill Drive
Beverly, MA 01915
USA
+1-855-737-9355
https://seqwell.com/