# NGS Library Quantification Product Handling Guide

| | |
|---|---|
| Shipping: | On Dry or Blue Ice |
| Catalog number: | MDX039 |
| Batch No.: | See vial |
| Concentration: | 2x |

**Store at –20 °C**

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**Storage and stability:**
NGS Library Quantification is shipped on dry or 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:**
Bioline operates under ISO 13485 Quality Management System. NGS Library Quantification and its components are extensively tested for activity, processivity, efficiency, heat activation sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

**Notes:**
This reagent has been manufactured under 13485 Quality Management System and is suitable for further manufacturing use as an IVD component.

## Description

NGS Library Quantification provides fast and robust quantification of Illumina-based next generation sequencing (NGS) libraries. It consists of FAST qPCR Mix (SYBR®-based), six pre-diluted DNA standards, ranging from 10 pM to 100 aM (to minimize pipetting errors), Primer Mix and Dilution Buffer.

The Kit contains sufficient reagents for the production of at least twenty DNA standard curves. This will allow the quantification of up to 18 DNA libraries on separate 96 or 384 well plates and up to a maximum of 62 libraries on six 96 well plates or 76 libraries on two 384 well plates.

### Kit Components

**Table 1.**

| Reagent |
|---|
| Primer Mix |
| FAST qPCR Mix, 2x |
| Dilution Buffer |
| DNA Standard 1 (10 pM) |
| DNA Standard 2 (1 pM) |
| DNA Standard 3 (100 fM) |
| DNA Standard 4 (10 fM) |
| DNA Standard 5 (1 fM) |
| DNA Standard 6 (100 aM) |

### User Guidelines

The 342 bp fragment used as a standard in this protocol (labelled DNA Standards 1-6) consists of a linear DNA fragment flanked by additional stabilizing DNA. It is highly recommended that no template controls (NTC) are included in each assay to detect contamination introduced during reaction set-up. NTC reaction should give a Ct values that are at least 3.5 cycles later that the average Ct value for Standard 6.

To help prevent any carry-over DNA contamination, we recommend that separate areas maintained for library preparation, reaction set-up, PCR amplification and any post-PCR gel analysis.

### Preparation

We recommend preparing a 1:10,000 diluted sample of the library using the Dilution Buffer. In order to improve the accuracy of the quantification, we suggest including additional dilution samples of each library (such as 1:100,000 and/or 1:1,000,000). Using two or more different dilutions of the library will ensure that at least one dilution falls within the dynamic range of the standard curve generated.

### Assay setup

Prepare reaction in Table 2. The volumes given below are based on a standard 20 µL final reaction mix and can be scaled accordingly. We recommend assaying each standard and library sample in triplicate.

**Table 2.**

| Reagent | Volume |
|---|---|
| FAST qPCR Mix, 2x | 10 µL |
| Primer Mix | 5 µL |
| Diluted library or DNA standard (1-6) | 5 µL |
| **Total volume** | **20 µL** |

### Reaction conditions

The qPCR conditions in Table 3 have been optimized for a number of platforms, however they can be varied to suit different machine-specific protocols.

**Table 3.**

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Polymerase activation | 95 °C | 2 min | 1 |
| Denaturation | 95 °C | 5 s | 35 |
| Annealing/Extension | 60 °C | 45 s* | |

*If the average library fragment size is larger than 500 bp, then increase annealing/extension time to 60 s.

### Analysis and quantification

Generation of a standard curve from the standard samples:
Correlate the concentration values of the different standard dilutions against their respective Ct (averaged from each triplicate) using data generated by qPCR. These data can be used to calculate the efficiency of the reaction by generating a standard curve. This can be obtained by plotting the average Ct value against log (concentration). Many qPCR machines generate this type of curve using the software provided by the manufacturer. The efficiency of the reaction is calculated using the following formula:

$$Efficiency\ (\%) = (10^{\left(\frac{-1}{a}\right)} - 1) \times 100$$

Where $a$ is the slope of the standard curve. The software provided with many qPCR machines often provides routines to display this value. Check that the reaction efficiency is between 90-110% for the DNA standards.

### Quantification of the library concentration:

From the Ct values obtained for the library sample dilutions, the library concentration is calculated using the standard curve following the formula, *where the concentration of the library* (L) *in picomolar is given by:*

$$L = 10^{\left[\left(\frac{Ct-b}{a}\right) x \left(\frac{342}{average\ fragment\ length}\right) x\ dilution\ factor\right]}$$

Where $a$ and $b$ represent the standard curve slope and y-intercept respectively. The formula above uses a fragment length for the standards of 342 bp.

### Sequence of the primers:

- F: 5'-AAT GAT ACG GCG ACC ACC GA-3'
- R: 5'-CAA GCA GAA GAC GGC ATA CGA-3'

### Technical Support

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


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