

MedixMDx Library Quant Kit for Illumina® Separate-ROX

Cat. No: MX5101

Product Information Leaflet

Description

The MedixMDx Library Quant Kit is a quantitative PCR (qPCR) kit for accurate quantification of adapter-ligated molecules to be used in equipment for NGS from Illumina®. qPCR is the most reliable method for such quantification because it specifically detects and quantifies templates suitable for library preparation and clustering. This kit includes five DNA standards for easy and precise quantification. It also includes MedixMDx qGreen Mix, which uses antibody-mediated hot-start activity to achieve a common start for all reactions. This is necessary for proper DNA library quantification.

The MedixMDx Library Quant Kit delivers consistent and reliable measurements across a wide range of samples, from GC-rich to AT-rich templates, and fragment sizes. In the NGS workflow, this kit should be used immediately before cluster generation. Failure to properly quantify the amount of DNA for the cluster can result in overloading or underloading the flow cell, both of which have negative consequences for NGS results and data. Please refer to your Illumina® NGS equipment for further information on the optimal amount of DNA to be loaded.

Kit components

| Component | *MX5101-10 100 rxn | *MX5101-50 500 rxn |
|--------------------------------------------|-----------------------|-----------------------|
| 2x MedixMDx qGreen Mix | 1 X 1 mL | 5 X 1 mL |
| 50 µM ROX additive | 1 X 200 µL | 1 X 200 µL |
| MedixMDx DNA Standards for Illumina® (1–5) | 30 µL each | 85 µL each |
| 10x Illumina® primers | 1 X 1 mL | 1 X 0.2 mL |
| 10x dilution buffer | 2 X 1.5 mL | 1 X 0.6 mL |

*Other pack sizes or bulk orders are available upon request.

Storage and shipment

Transport with an ice pack or on dry ice (for shipments taking more than 2 days). The reagents should be stored between -30°C and -15°C upon arrival. The reagents are stable for 12 months if stored correctly. The reagents are stable for 1 month at 4°C.

Reaction mastermix set-up

The recommended mastermix set-up for a 20 µL reaction volume is shown in the table below.

| *Reagent | Volume (µL) | Final concentration |
|--------------------------------|-------------|-----------------------|
| 2x MedixMDx qGreen Mix | 10 | 1x |
| 10x Illumina® primers | 2 | 400 nM each P5 and P7 |
| Diluted sample or DNA standard | 4 | Variable |
| Nuclease-free water | 4 | |
| Total volume | 20 µL | |

*If your qPCR instrument requires ROX correction, add ROX additive to the mastermix at a final concentration of 500 and 50 nM for HI-ROX and LOW-ROX instruments, respectively.

Instrument and program set-up

| Number of cycles | Step | Temperature | Time |
|---------------------------------|-----------------------|-------------|--------|
| 1 | Polymerase activation | 95°C | 1 min |
| 40 | Denaturation | 95°C | 15 sec |
| | *Annealing/extension | 63°C | 45 sec |
| Optional melting curve analysis | | | |

Analysis

Using the Ct values obtained for the different DNA standards, draw a standard linear regression plot to calculate the efficiency, which should not be below 90%.

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Use the standard curve to accurately calculate the DNA concentration of your sample dilutions. Use the following equation to revert to the undiluted sample concentration:

Concentration of library = concentration for the reaction X dilution factor X (452 / average length of fragments).

Technical information and support

For technical enquiries or assay development support, please contact us via email at: mdx@medixbiochemica.com. Additional information and technical resources are available on our website at: www.medixbiochemica.com/en/MedixMDx.