sparQ HiFi PCR Master Mix

Cat. No. 95192-050 Size: 50 reactions **Store at -25°C to -15°C**
95192-250 250 reactions

Description

The sparQ HiFi PCR Master Mix is a high efficiency, high-fidelity, and low bias PCR master mix for NGS workflows requiring DNA library amplification prior to sequencing. The included primer mix allows amplification of DNA libraries flanked by adapters containing the P5 and P7 Illumina® flow cell sequences.

Components

	Volume	
Component Description	**95192-050**	**95192-250**
HiFi PCR Master Mix (2X)	1 x 1.25 ml	5 x 1.25 ml
Primer Mix	1 x 0.150 ml	5 x 0.150 ml
HiFi Enhancer*	1 x 0.150 ml	5 x 0.150 ml

* The optional HiFi enhancer can be used for optimizing workflows requiring amplification of libraries immobilized on magnetic beads

Storage and Stability

Store components in a constant temperature freezer at -25°C to -15°C upon receipt.
For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

General Guidelines

- Use good laboratory practices to minimize cross-contamination of nucleic acid products.
- Always use PCR tubes, microfuge tubes, and pipette tips that are certified sterile, DNase- and RNase-free.
- Wipe down work areas and pipettes with an RNase and DNA cleaning product.
- Thaw reagents on ice. Once thawed, finger flick (do not vortex) the tube containing HiFi PCR Master Mix to ensure even distribution of contents. Other tubes can be briefly vortexed to ensure mixing.
- For consistent library amplification, ensure the thermal cycler used in this protocol is in good working order and has been calibrated to within the manufacturer's specifications.
- Briefly centrifuge tubes prior to opening to avoid loss of material.
- Read the entire protocol before beginning. Take note of stopping points where samples can be frozen at -20°C and plan your workflow accordingly

Point in protocol where procedure can be stopped and stored at appropriate conditions outlines

Take note of recommendations in protocol

Use caution to obtain the best results when performing protocol

Additional required reagents and materials that are not supplied

- Purification Beads
  Purification beads for post-amplification reaction cleanups are not included with the kit and must be purchased separately. This protocol has been validated using both 1X sparQ PureMag Beads and AMPure® XP for post-amplification reaction cleanup. AMPure XP is available from Beckman Coulter, Inc.. sparQ PureMag Beads can be purchased from Quantabio.

- Nuclease-free Water
  Any commercially available nuclease-free, molecular biology grade water can be used with this kit. Do not use DEPC-treated water.

- 80% Ethanol
  A fresh solution of 80% ethanol should be prepared and stored at room temperature immediately prior to the reaction cleanup steps.

Protocol

Routine Library Amplification

Library amplification is generally recommended if the input DNA for library construction is below 100 ng. The PCR reagents (HiFi PCR Master Mix and Primer Mix) can be used for high-fidelity amplification of the DNA library. The Primer Mix contains both forward and reverse primers and is compatible with libraries flanked by the standard P5 and P7 adapter sequences. If a different primer mix is preferred, please follow the supplier's instructions.

1. Prepare the PCR reaction in a separate tube on ice by combining the HiFi PCR Master Mix (2X) and Primer Mix per the table below. Mix well by pipetting. Volumes can be scaled as needed for the desired number of reactions.

Components	Volume for 1 Reaction (µl)
HiFi PCR Master Mix (2X)	25
Primer Mix	1.5
Total	**26.5**

2. Add 26.5 µl of the master mix to 23.5 µl of adapter-ligated DNA library sample* in a thin-walled PCR tube and mix gently by pipetting up-and-down 8 -10 times. Keep the PCR tube on ice during reaction setup.

** It is highly recommended to perform a post-ligation cleanup prior to library amplification to remove unligated adapters and adapter dimers from the reaction.*

3. Program a thermal cycler with the parameters listed in the table below. Set the instrument's heated lid to 105°C. When the thermal cycler block reaches 98°C, pause the program.

Step	Temperature	Incubation Time	Cycles
1	98°C	2 min	1
2	98°C	20 sec	Varies based on input amount – see table below
3	60°C	30 sec	
4	72°C	30 sec	
5	72°C	1 min	1
6	4°C	Hold	1

Note: *Excessive library amplification increases the likelihood of amplification bias and the generation of unwanted artifacts. Therefore, it is recommended to limit the number of amplification cycles to the minimum needed to achieve acceptable yield for downstream processes. Yields in the range of 250 – 1000 ng are typically sufficient for target capture and sequencing applications.*
The table below provides guidelines on the number of cycles to yield 500 ng of DNA library from various sample input amounts.

Input DNA sample (ng)*	Suggested number of cycles to yield 500 ng of DNA library
1000	0 - 1
500	1 - 2
100	4 - 5
50	5 - 6
10	8 - 10
1	13 - 15
0.25	16 - 18

**DNA Library was prepared using sparQ Library Prep Kit prior to amplification.*

4. Pulse-spin the sample tube and immediately transfer to the pre-heated thermal cycler (98°C). Resume the cycling program.
5. When the thermal cycler program is complete and sample block has returned to 4°C, remove the sample from the block and proceed immediately to post-amplification cleanup using purification beads.

Post-Amplification Cleanup

This protocol has been validated using both 1X sparQ PureMag Beads and AMPure XP for post-amplification reaction cleanup. Conditions may differ if other beads are used.

6. Equilibrate the sparQ PureMag Beads to RT for 20 min.
7. Thoroughly vortex the sparQ PureMag Beads slurry and add 50 µl (1X) to the PCR reaction. Mix well by pipetting.
8. Incubate the mixture for 5 min at RT. Pellet the beads on a magnetic stand (e.g., DynaMag™) and carefully discard the supernatant.
9. Wash the beads with 200 µl of 80% ethanol. Pellet the beads on the magnetic stand and discard the supernatant. Repeat the wash once.
10. Air-dry the beads on the magnetic stand for 5 - 10 min or until the beads are dry. Over-drying of beads may result in lower DNA recovery.
11. Resuspend the dried beads in 32.5 µl of 10 mM Tris-HCl, pH 8.0 to elute. Pellet the beads on the magnetic stand. Carefully transfer 30 µl of supernatant into a new tube. The sample can be stored at -20°C if not proceeding immediately to library quantification or other downstream processes.

Protocol

Post-capture PCR enrichment

The sparQ HiFi PCR Master Mix is compatible with target enrichment workflows such as those utilizing bead-based hybridization capture probes and panels. The following instructions are validated for PCR amplification of targets enriched using xGen® Lockdown® Probes and reagents from IDT. In this workflow, the targets to be amplified are immobilized on Dynabeads® M-270 Streptavidin beads.

1. Prepare the post-capture amplification in a separate tube per the table below.

Components	Volume for 1 Reaction (µl)
HiFi PCR Master Mix (2X)	25
Primer Mix *	0.75
HiFi Enhancer †	2
Nuclease-free water	2.25
Beads with captured DNA template ‡	20
Total	**50**

** A final concentration of 0.5 µM each primer for on-bead amplification. Please follow manufacturer instructions if using primers other than those supplied with the sparQ HiFi Master Mix.*

† 2 - 2.5 µl of the HiFi Enhancer should be added to each reaction to ensure high efficiency on-bead amplification. When using a higher volume of beads, scale the amount of HiFi Enhancer accordingly.

‡ *The xGen Lockdown Probe hybridization protocol from IDT uses 100 µl worth of M-270 beads for capture with a final resuspension in 20 µl of nuclease-free water.*

2. Pipette up and down to ensure mixing of components. Spin down gently, but ensure the beads remain in solution.

3. Place the tube in a thermal cycler with the instrument's heated lid set to 105°C. Program and run the reaction per the parameters listed in the table below.

Step	Temperature	Incubation Time	Cycles
1	98°C	45 sec	1
2	98°C	15 sec	12
3	60°C	30 sec	
4	72°C	30 sec	
5	72°C	1 min	1
6	4°C	Hold	1

Post-Amplification Cleanup

4. Equilibrate the sparQ PureMag Beads to RT for 20 min.
5. Thoroughly vortex the sparQ PureMag Beads slurry and add 75 µl (1.5X) to each PCR reaction. Mix well by pipetting.
6. Incubate the mixture for 5 min at RT. Pellet the beads on a magnetic stand and carefully discard the supernatant.
7. Wash the beads with 200 µl of 80% ethanol. Pellet the beads on a magnetic stand and discard the supernatant. Repeat the wash once.
8. Air-dry the beads on the magnetic stand for 5 – 10 min or until the beads are dry. Over-drying of beads may result in lower DNA recovery.

9. Resuspend the dried beads in 22 µl of 10 mM Tris-HCl, pH 8.0 to elute. Pellet the beads on the magnetic stand. Carefully transfer 20 µl to a new tube.
   The sample can be stored at -20°C for up to 1 week.

Protocol

Library Validation and Quantification

DNA libraries constructed using either of the above two protocols should be validated and quantified to ensure optimal input for sequencing reactions.

Average fragment length can be measured using a digital electrophoresis system such as the Agilent 2100 Bioanalyzer or Agilent 2200 TapeStation per manufacturer instructions.

An estimate of library concentration can be assessed using Qubit™ or another fluorometric method.

More accurate library quantification can be obtained using a qPCR-based assay. Quantabio offers the PerfeCTa® NGS Library Quantification Kits (95155-500, 95156-500) for accurate quantification of DNA library molecules suitable for sequencing on Illumina NGS platforms.

Quality Control

Quality of the 2X HiFi PCR Master Mix is tested functionally by amplification of a DNA library prepared from mixed bacterial genomic DNA with GC-content of 10-80%. The differences in library yield and profile among different lots must not exceed 15%. Sequencing of the amplified library must yield mapped reads >90% and normalized coverage between 0.7 and 1.3 across the full GC spectrum.

Limited Label Licenses

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this manual and for use with components contained in the kit only. Quantabio, LLC. grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this manual, and additional protocols available at www.quantabio.com. Some of these additional protocols have been provided by Quantabio product users. These protocols have not been thoroughly tested or optimized by Quantabio, LLC. Quantabio, LLC. neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, Quantabio, LLC. makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. Quantabio, LLC. specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. Quantabio, LLC. may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

Quantabio products are manufactured in Beverly, Massachusetts, Frederick, Maryland and Hilden, Germany.
Intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

Trademarks

AMPure® XP is a registered trademark of Beckman Coulter. DynaMag™, Dynabeads® and Qubit™ are trademarks of Thermo Fisher Scientific and its subsidiaries. Unless otherwise specified, xGen® and Lockdown® are registered trademarks of Integrated DNA Technologies, Inc. Illumina is a registered trademark of Illumina, Inc. PerfeCTa is a registered trademark of Quantabio, LLC.

Related NGS Products Sold Separately

sparQ DNA Frag & Library Prep Kit

Cat. No.	95194-024	Size:	24 reactions
	95194-096		96 reactions

The sparQ DNA Frag & Library Prep Kit is optimized for enzymatic fragmentation of DNA and streamlined construction of high quality libraries for sequencing on Illumina® NGS platforms. The simple, convenient 2-step workflow can be completed in 2.5 hours with minimal hands-on time and accommodates DNA input amounts from 1 ng to 1000 ng.

sparQ DNA Library Prep Kit

Cat. No.	95191-024	Size:	24 reactions
	95191-096		96 reactions

The sparQ DNA Library Prep Kit is optimized for the rapid construction of DNA libraries from fragmented double-stranded DNA for sequencing on Illumina® NGS platforms. The simplified protocol speeds up library prep to 2.5 hours with minimal hands-on time and accommodates DNA input amounts from 250 pg to 1 µg.

sparQ PureMag Beads

Cat. No.	95196-005	Size:	5 ml
	95196-060		60 ml
	95196-450		450 ml

sparQ PureMag Beads is a fast and reliable nucleic acid purification system for reaction cleanup and size selection in NGS workflows. It can be used to quickly remove primers, primer-dimers, unincorporated nucleotides, salts, adapters and adapter-dimers from NGS library prep reactions to improve downstream sequencing performance.

sparQ Universal Library Quant Kit

Cat. No.	95210-100	Size:	100 reactions
	95210-500		500 reactions

The sparQ Universal Library Quant Kit is optimized for rapid, sensitive, and accurate quantification of NGS libraries of various sizes and GC-contents. The kit uses fast cycling protocol, allowing results to be achieved in 40 minutes versus 1 hour and 20 minutes with other NGS library quantification kits.