AVICENNA

AviSeq™

Genomic DX

Barcode Set 17-32

REF: AVG503 96 TESTS

USER GUIDE

AVG503_IFU_Rev00_MAY22

CE

IVD

CONTENTS

1. PRODUCT APPLICATIONS

Aviseq Barcode Set 17-32 provided in this kit must be used in combination with the AviSeq range of kits to prepare libraries for next-generation sequencing on the Ion Torrent range of sequencing instruments.

2. KITS CONTENTS

Aviseq Barcode Set 17-32 contain ready-to-use reagents for the incorporation of the Barcodes and Adapters to DNA libraries rendering them suitable for analysis on Ion Torrent instruments. The PCR product from Step 1 of the Target Enrichment protocol is the starting material (DNA) for this PCR, in which Barcodes and Adapters are added to the PCR products to produce a library suitable for NGS on the Ion Torrent instruments only.

Aviseq BARCODE SET 17-32 (cod. AVG503)			
TUBE	**VOLUME**	**TUBE**	**VOLUME**
Barcode 17	24 µl	Barcode 25	24 µl
Barcode 18	24 µl	Barcode 26	24 µl
Barcode 19	24 µl	Barcode 27	24 µl
Barcode 20	24 µl	Barcode 28	24 µl
Barcode 21	24 µl	Barcode 29	24 µl
Barcode 22	24 µl	Barcode 30	24 µl
Barcode 23	24 µl	Barcode 31	24 µl
Barcode 24	24 µl	Barcode 32	24 µl

Table 1: Content of Aviseq Barcode Set 17-32 kit.

3. STORAGE AND STABILITY

All reagents provided in this kit are ready to use and must be stored at -20°C.
A high standard of performance capacity and quality will be maintained until the expiry data marked on each reagent tube and on the external jar/container if the kit remains intact, is properly stored and undergoes a maximum of 3 freeze-thaw cycles.
When the reagents are thawed they must be kept on ice throughout the process and returned to -20°C storage immediately after use.

4. REQUIRED MATERIAL NOT INCLUDED

4.1 Generic Material

- Computer with constantly updated and guaranteed secure internet connection
- Micropipettes calibrated and periodically verified 0.2-2 µl, 2-20µl, 20-200µl or 100-1000µl and filter tips
- Vortex
- Disposable Gloves without powder
- Thermal cycler calibrated and periodically verified
- Tubes and Caps or 96-wells plate, as needed, DNase and RNase free
- Nuclease-free water
- 1,5 ml tube magnetic separator or 96-wells plate compatible magnetic separator
- Fresh 70% ethanol and 5% hypochlorite
- Ion Torrent sequencer calibrated and periodically verified

4.2 Specific Material

The material listed below has been used and validated for use in this procedure:

- Qubit™ 2.0 Fluorometer (Invitrogen Cod. Q32866) or Qubit™ 3.0 Fluorometer (Invitrogen Cod. Q33216) or Qubit™ 4.0 Fluorometer (Invitrogen Cod. Q33226) calibrated and periodically verified
- Qubit™ assay tubes (Invitrogen Cod. Q32856)
- Qubit™ dsDNA HS Assay Kit (Invitrogen, cod. Q32851)
- Magnetic beads for the purification of genomic libraries, AMPure XP (Beckman Coulter, cod. A63880)

QUALITATIVE ANALYSIS OF DNA (Optional)

- Agilent 2100 Bioanalyzer system with DNA reagent kit calibrated and periodically verified

5. IMPORTANT NOTES AND SAFETY INFORMATION

Users must comply with the recommendations listed below and failure to do so will result in suboptimal outcomes or failed runs. If the kit or any component thereof is forwarded to a third party, it is the your responsibility to provide the end user with these recommendations. Note: the manufacturer is committed to the highest level of quality assurance at all steps in the procedure and will provide full support to all customers.

- This kit intended for use by professionals trained in molecular biology. Use by untrained individuals may result in suboptimal outcomes.
- Do not use if package damaged
- Biological samples and all reagents should be used in properly equipped rooms, clean and clear of potential contaminants. We highly recommend cleaning working areas frequently using a solution containing sodium hypochlorite 1-5% followed by sterile water and 70% Ethanol.
- Always use appropriate personal protective equipment (PPE) such as laboratory coat, gloves and safety goggles during all steps described in the protocol.
- Ensure that you are aware of all safety instructions associated with all equipment and related electrical supply, chemicals and other resources utilized in the performance of the procedures outlined in this IFU.
- Ensure that quality control procedures are in place at each phase of the protocol are adequate to detect any errors, particularly after each usage of the kit. This should include a quality evaluation of the results generated. Contact your local supplier immediately on detecting any errors and/or anomalies in the performance of the kit.
- It is essential in a diagnostic and/or clinical setting that users of this kit put strong control and quality assurance procedures in place to minimize or eliminate diagnostic errors following a critical evaluation of possible risk factors in the protocol. Any errors detected must be reported to the associated medical staff immediately.
- To avoid contamination of reagents we recommend using DNase/RNase free tubes, filter tips and to pay particular attention to keep all instruments clean and free of contaminants.
- We highly recommend the lab is designed in a unidirectional workflow from the initial phase of DNA isolation following the PCR preparation phase, amplification and post-amplification phases in order to keep working areas separated for the different phases of the procedure. Each area must have dedicated laboratory equipment (e.g., vortex, pipettes, etc.,), consumables (e.g. tubes, pipette tips, etc.,) and PPE (e.g., gloves, etc.,).
- Used reagents and biological samples must be wasted according to local regulations and legal procedures.

6. PROTOCOL

For detailed instructions on the use of the barcodes provided in this kit, please refer to the instructions for use of the the AviSeq kit you are utilizing.

7. TROUBLESHOOTING

PROBLEM	POSSIBILE CAUSE	SUGGESTION
Absence of bands on agarose gel after electrophoresis	Incorrect thermocycling profile	Verify the PCR thermal profile and calibration then repeat the PCR reaction
	Mistakes in master mix preparation	Verify PCR mix components and repeat the PCR reaction
	Degraded reagents	Verify expiry date and storing conditions of the products
	Presence of inhibitors	Verify concentration and quality of DNA extracted using a spectrophotometer. If necessary, repeat DNA extraction.
	Low amount of DNA	Verify concentration and quality of DNA extracted using a spectrophotometer. If necessary, repeat DNA extraction.
Absence of bands on agarose gel after electrophoresis	Wrong PCR machine settings	Verify the PCR thermal profile and calibration then repeat the PCR reaction
Presence of fragments with low molecular weight	Mistakes in master mix preparation	Verify PCR mix components and repeat the PCR reaction

8. SYMBOLS

CE	*According to 98/79/CE Directive*		*Catalogue number*
	In Vitro Diagnostic Medical Device		*Batch code*
	Expiration date		*Temperature limitation*
	Consult instruction for use (IFU)		*Sufficient for n. tests*
	Manufacturer		*Do not use if package damaged*

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