

Index Set 384

REF: AVG405 384 TESTS



USER GUIDE

AVG405_IFU_Rev00_MAY22









CONTENTS

CONTENTS	2
1. PRODUCT APPLICATIONS	
2. KITS CONTENTS	
3. STORAGE AND STABILITY	
4. REQUIRED MATERIAL NOT INCLUDED	
5. IMPORTANT NOTES AND SAFETY INFORMATION	
6. PROTOCOL	
7. TROUBLESHOOTING	
8. SYMBOLS	







1. PRODUCT APPLICATIONS

Index Set 384 kit must be used in combination with AviSeq panel kits. The intended use of those kits is the generation of a library suitable for NGS analysis with Illumina sequencing instruments.

2. KITS CONTENTS

Index Set 384 kit contain ready-to-use reagents for the insertion of the adapters specific for Illumina instruments. The starting DNA is from the PCR product obtained in the step 1 of target enrichment. The result of this second PCR is a library suitable for Next Generation Sequencing analysis with Illumina sequencing instruments.

Index Set 384 (Cod. AVG405)				
PLATE	QUANTITY			
А	1 (96 WELL)			
В	1 (96 WELL)			
С	1 (96 WELL)			
D	1 (96 WELL)			

Table 1: Content of the Index Set 384 kit.

3. STORAGE AND STABILITY

All reagents provided with our kits are ready to use and should be stored at -20°C.

The kit, intact and properly stored, will maintain high quality performance capacity until the expiry date marked on each plate and on the external jar/container.

When the reagents are thawed they must be kept on ice throughout the process.

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 cyclercalibrated and periodically verified
- Tubes and Caps or 96-wells plate, as needed, DNase and RNase free
- Nuclease-freewater
- 1,5 ml tube magnetic separatoror 96-wells plate compatible magnetic separator
- Fresh 70% ethanol







4.2 Specific Material

The materiallisted below has been used in the validation of this kit:

- Qubit[™] 2.0 Fluorometer(InvitrogenCod. Q32866) or Qubit[™] 3.0 Fluorometer(InvitrogenCod. Q33216) or Qubit[™] 4.0 Fluorometer(InvitrogenCod. Q33226) calibrated and periodically verified
- Qubit[™] assay tubes (InvitrogenCod. Q32856)
- Qubit[™] dsDNA HS AssayKit (Invitrogen,cod. Q32851)
- Magnetic beads for the purification of genomic libraries, AMPure XP (Beckman Coulter, cod. A63880) or MAGTIVIO (magSi-NGSPREPPlus, cod. MDKT00010075)
- Illuminasequencer calibrated and periodically verified
- AviSeq panel kit

QUALITATIVE ANALYSIS OF DNA (Optional)

· Agilent 2100 Bioanalyzer systemwith DNA reagentkit calibrated and periodically verified

5. IMPORTANT NOTES AND SAFETY INFORMATION

The user is required to apply the following provisions.

If the device or the results it generates, even in part, is transferred to a third party, the user must inform the end-user about the application of the specific provisions. The manufacturer is committed to constantly checking the possibilities of implementing the procedures, providing support to users.

- The kit is for professionaluse, it must be used by trained professionalsin molecular biology.
- 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 suggest cleaning working areas frequently using a solution containing sodium hypochlorite
- Always use safety equipment such as laboratory coat, gloves and safety goggles during all steps described in the protocol.
- Check the risks and safety procedures associated with instruments, electricity, chemicals and other resources applied to the use of the device.
- Prepare ways of detecting errors in the operation of the device, evaluating after each usage the quality of the results generated; in case of doubts or anomalies found, the supplier must be promptly contacted for support.
- When the results produced are used in diagnostic or clinical processes, the user is required to consider the possible risks associated with diagnostic errors, to set up control mechanisms and to inform the medical personnel responsible for the diagnostic or clinical processes.
- 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 suggest preparing 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 using for each phase dedicated laboratory coats, micropipettes, tubes and filter tips.
- Used reagents and biological samples must be wasted according to legal procedures.

6. PROTOCOL

For the operative procedure please refer to the manual provided with the AviSeq kits.







7. TROUBLESHOOTING

PROBLEM	POSSIBILE CAUSE	SUGGESTION	
Absence of bands on agarose gel after electrophoresis	Wrong PCR thermal profile	Verify the PCR thermal profile and calibration ther 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.	
Presence of fragments with low molecular weight	Primer residues and / or degradation of adapters, primers dimers, etc.	Eliminate low molecular weight fragments by purification with AMPure XP Beads	

8. SYMBOLS

C€	According to 98/79/CE Directive	REF	Catalogue number
IVD	In Vitro Diagnostic Medical Device	LOT	Batch code
	Expiration date	1	Temperature limitation
i	Consult instruction for use (IFU)	$\overline{\Sigma}$	Sufficient for n. tests
	Manufacturer		Do not use if package damaged



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