DeepChek® Assay

13-Plex KB Drug Susceptibility Testing

V1.x *(RUO)*

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User Guide

Version 1 – Revision 0

For Research Use Only (RUO). Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.

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Document control

Date	Device version	IFU version	Description of change
21/04/2022	A	1.0.0	Document creation
27/04/2022	A	1.0.1	Document revision – small edits; review of table 1 with anti-TB drugs acronyms; revision of tube label volumes in table 2; add the figure 1.

Contents

Application

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The ***DeepChek® Assay 13-Plex KB Drug Susceptibility Testing V1.x (RUO)*** is a polymerase chain reaction (PCR) test (nucleic acid technique (NAT)) intended to screen mutations on targeted regions of the Mycobacterium tuberculosis (KB) genome.

The test is amplifying thirteen (13) genes of Mycobacterium tuberculosis specimens which harbor mutations described as sufficient, when present, to determine level of resistance to anti-tuberculosis (TB) drugs.

The ***DeepChek® Assay 13-Plex KB Drug Susceptibility Testing V1.x (RUO)*** is intended for use by trained laboratory personnel specifically instructed and trained in the techniques of PCR and next generation sequencing (NGS) workflows.

Principles of the assay

The ***DeepChek® Assay 13-Plex KB Drug Susceptibility Testing V1.x (RUO)*** is a polymerase chain reaction test which includes primers, reverse and forward, designed to amplify Mycobacterium tuberculosis from extracted DNA specimens.

During each round of thermal cycling, amplification products dissociate to single strands at high temperature allowing primer annealing and extension as the temperature is lowered. Exponential amplification of the product is achieved through repeated cycling between high and low temperatures, resulting in a billion-fold or greater amplification of target sequences.

The ***DeepChek® Assay 13-Plex KB Drug Susceptibility Testing V1.x (RUO)*** is performed on a PCR instrument.

Subsequently, the amplicons can be used for next generation sequencing (NGS) and analyzed with a downstream analysis software to list in a report TB genome mutations according to available public reference knowledge databases and to facilitate the study of the relationship between mutations and resistance to anti-TB drugs.

Anti-tuberculosis drugs (acronyms)		**Targeted genes by the DeepChek® Assay 13-Plex KB Drug Susceptibility Testing**
1st line	rifampicin (RMP)	rpoB
	isoniazid (INH)	up/mabA,inhA ; katG ; furA-katG intergenic region
	pyrazinamide (PZA)	pncA
	ethambutol (EMB)	embB
2nd line	Fluoroquinolones (moxifloxacin (MOX), levofloxacin (LFX))	gyrA, gyrB
	amikacin (AMK)	rrs
	kanamycin (KAN)	Eis ; up eis ; rrs
	capreomycin (CAP)	tlyA ; rrs
	streptomycin (STR)	rrs ; rpsL
	ethionamide (ETH)	inhA
New antibiotics	bedaquiline (BDQ) clofazimine (CFZ)	rv0678

Table 1: list of anti-TB drugs and related genes targeted by the DeepChek® Assay 13-Plex KB Drug Susceptibility Testing (V1.x) (RUO)

Assay components

Required and supplied by ABL

The ***DeepChek® Assay 13-Plex KB Drug Susceptibility Testing (V1.x)*** (RUO) (REF 128A24) is provided for 24 reactions.

Label	Volume for 24 Rxn.	Color cap	Storage
Master Mix	660 µL	Green	-25°C to - 15 °C
Primers Mix	440 µL	Pink	-25°C to - 15 °C

Table 2: Reagents composing the DeepChek® Assay 13-Plex KB Drug Susceptibility Testing (V1.x) (RUO)

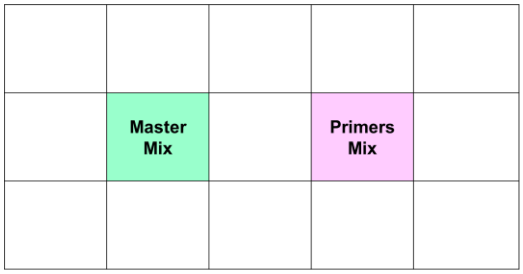

Figure 1: Mapping of assay components by color cap

The ***DeepChek® TB Sample Preparation (150 mL)*** (CE-IVD) (REF 127B-240862) enables the preparation of 150 mL of solution for decontaminating and concentrating tuberculosis sample (sputum) sediments.

Label	Format	Storage
MycoPrep™ Reagent	Package * 2	Room temperature
Phosphate Buffer	Ampule in a bottle * 1	Room temperature

Table 3: Reagents composing the DeepChek® TB Sample Preparation (150 mL)

Reagent transport, storage and handling

The ***DeepChek® Assay 13-Plex KB Drug Susceptibility Testing (V1.x) (RUO)*** is shipped with dry ice and shall be maintained and stored at - 25°C to - 15 °C to avoid compromising cold chain integrity.

The sample preparation kit, ***DeepChek® TB Sample Preparation*** *(150 mL),* is shipped at room temperature.

Products are stable until the expiration date stated on the label. Note the production date and expiration date listed on the label. Reagents from different lot numbers should not be mixed. Note: Multiple thaw-freeze cycles should be avoided. Aliquoting should be considered.

Materials required but not provided

Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations. Please refer to relevant manufacturer's Instructions for Use (IFU) to proceed with the instrument.

DNA extraction and purification of clinical specimen

Any laboratory validated instrument for DNA extraction and purification using magnetic-bead technology shall work with the test. The following nucleic acid extraction and purification kits were tested successfully for use with this test.

Supplier	Equipment, Materials and Reagents
Roche Diagnostics	MagNa Pure 24 instrument Software version v1.1 Catalog #07290519001B
Roche Diagnostics	MagNA Pure 24 Total NA Isolation Kit Catalog #07658036001
Roche Diagnostics	MagNA Pure Tube 2.0mL Catalog #07857551001
Roche Diagnostics	MagNA Pure 24 ProcessingTip Park/Piercing Tool Catalog #07345585001
Roche Diagnostics	MagNA Pure Sealing Foil Catalog #06241638001
Roche Diagnostics	MagNA Pure Bacteria Lysis Buffer Catalog #04659180001
Roche Diagnostics	MagNA Pure 24 Processing Cartridge Catalog #07345577001

Table 4: Tested Equipment, Materials and Reagents for DNA extraction and purification

Supplier	Equipment, Materials and Reagents
Magtivio B.V.	PurePrep 96 Nucleic Acid Purification Instrument Software version 1.0.9a Catalog #AS00001
Magtivio B.V.	MagSi-NA Pathogens Catalog #MDKT00210960
Magtivio B.V.	2 mL Deep-well Plate with square wells for PurePrep 96 Instrument Catalog #MDPL00200060
Magtivio B.V.	200 µL square-well Elution Plate for PurePrep 96 Instrument Catalog #MDPL00190060
Magtivio B.V.	96 well Tip-Comb for PurePrep 96 Instrument Catalog #MDPL00210060
Roche Diagnostics	MagNA Pure Bacteria Lysis Buffer Catalog #04659180001

Table 5: Tested Equipment, Materials and Reagents for DNA extraction and purification

Instruments

Any laboratory validated thermal cycler with enough ramp rate of ≥ 1°C/s shall be sufficient (i.e., ThermoFisher Scientific ProFlex™ PCR System (model 3 x 32-well (Catalog #4484073) or 96-well (Catalog #4484075) and associated specific material).

Master mix preparation

- Benchtop centrifuge with rotor for 0.5 mL/1.5 mL reaction tubes (able to reach 10,000 rpm).
- Benchtop vortex mixer.
- Microliter pipets dedicated to PCR (0.1-2.5 µL; 1-10 or 1-20 µL; 20-200 µL; 1000 µL).
- Pipetting Robot (optional).
- Nuclease-free aerosol-resistant sterile PCR pipet tips with hydrophobic filters.
- Adjustable pipettes & fitting filtered pipette tips.
- Appropriate PPE & workspaces for working with potentially infectious samples.
- Surface decontaminants such as DNAZap (Life Technologies), DNA Away (Thermo Fisher Scientific), RNAse Away (Thermo Fisher Scientific), 10% bleach.
- Nuclease-free H_2O.
- 0.5 ml or 1.5 ml RNase- and DNase-free PCR tubes.
- Ice/Icebox or even cooling blocks.

- 32 well or 96 well plate cooler (optional).
- 32 well or 96 well PCR plates.
- Plate thermo seals.
- Plate centrifuge.
- 0.2 mL thin walled 8 tube & domed cap.

Amplicons quality control

Instruments and reagents can be used for the quality control of amplicons (i.e., capillary electrophoresis (i.e., Agilent ScreenTape D1000 and Reagents D1000 for Agilent TapeStation 4150 (reagent)); DNA sample electrophoresis (i.e., Invitrogen E-Gel EX reagents for 0.8–2% agarose gel in 0.5x TBE electrophoresis buffer)).

Warnings and precautions

- **For Research Use Only (RUO). Not for use in diagnostic procedures.** No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.
- This product has been tested only for nucleic acid from *Mycobacterium tuberculosis*, not for any other tuberculosis or pathogens.
- Handle all specimens as infectious using safe laboratory procedures.
- Laboratories maybe required to report all test results to the appropriate public health authorities.
- Store assay reagents as indicated on their individual labels.
- Do not mix reagents from different kit lots.
- Reagents must be stored and handled as specified in these instructions for use. Do not use reagents past expiration date.
- Work surfaces and pipettes should be cleaned and decontaminated with cleaning products such as 10% bleach, “DNAZap™” or “RNase AWAY®” to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- When a positive control is used, it should be handled in an area separate from sample receiving, accessioning and processing areas to avoid contamination of the samples with amplifiable material.
- Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and specimens are handled.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- Dispose of waste in compliance with the local, state, and federal regulations.
- Frequent cleaning of the wells of the PCR instrument plate is recommended to prevent contamination.
- To avoid contamination, use separated and segregated working areas: 1) Reagent preparation area – preparing the reagents for amplification, 2) Dilution of positive control material according laboratory guidelines, 3) sample preparation area- isolation of the RNA/ DNA from sample and control, and 4) Amplification area- amplification and detection of nucleic acid targets.
- Check whether the PCR reaction tubes are tightly closed before loading on the PCR instrument to prevent contamination of the instrument from leaking tubes.

Workflow

The following diagram illustrates the complete workflow, from sample to analysis report.

The total time for 24 samples is about 29 hours.

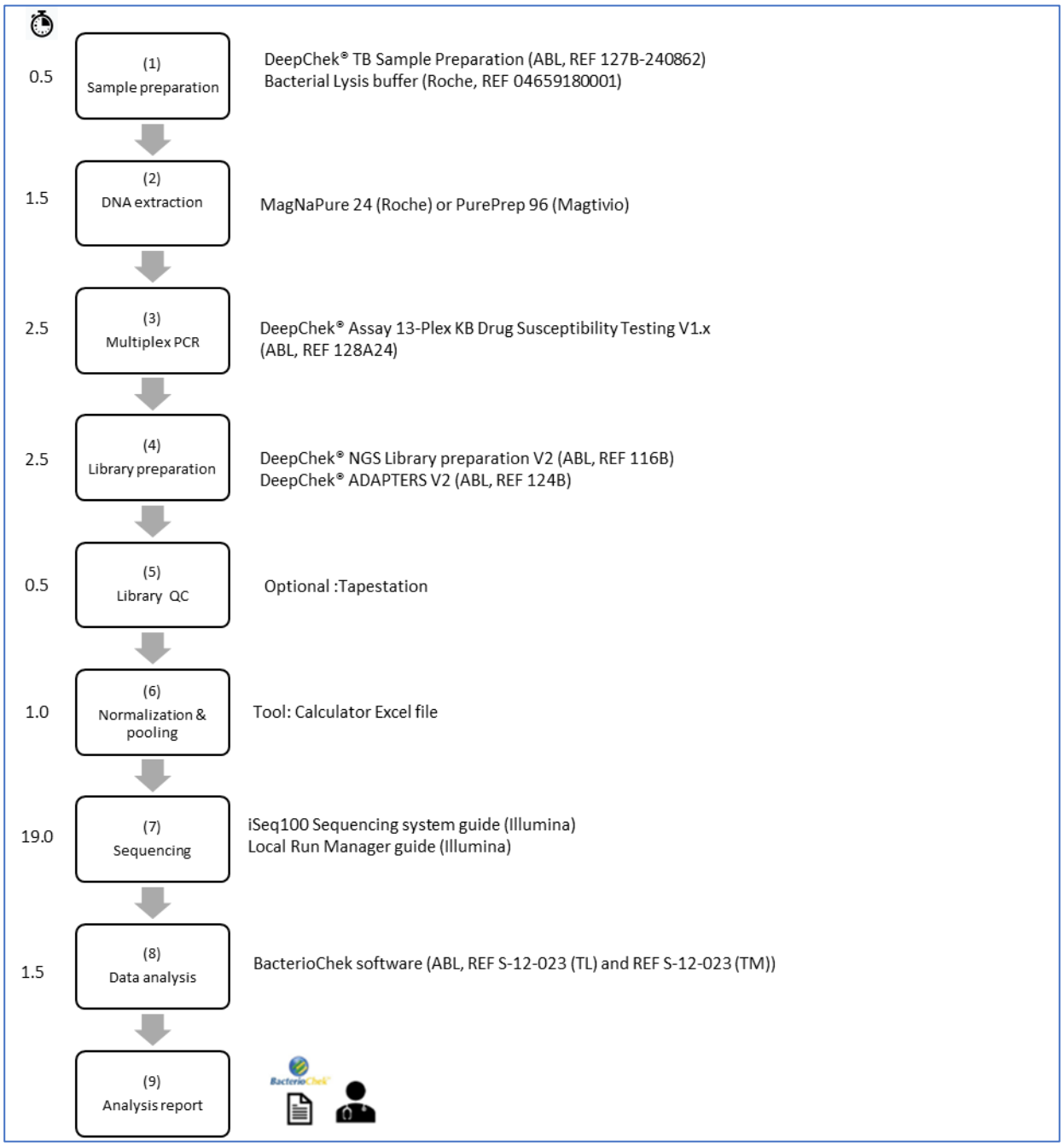

Figure 2: Sample to analysis report workflow with processing time and main references.

DNA Sample preparation & Extraction

Reagent preparation

- Prepare MycoPrep™ Phosphate Buffer (REF 127B-240862) as needed, by dispensing the whole content into a 500 ml volumetric flask and fill to line with distilled or deionized water.
- Check the pH with a pHmeter. The pH of the solution should be 6.8.

- Transfer the buffer solution to a screwcapped container and, with cap loosened, autoclave at 121°C for 15 min.
- Cool to room temperature and tighten the cap.
- Be careful not to spill the content, then loosen screw-cap on the MycoPrep™ Reagent bottle (REF 127B-240862).
- Locate ampule in bottle, squeeze excess air from the bottle and tighten the cap.
- With the bottle in the upright position, squeeze it until the ampule breaks.
- Shake gently to dissolve. Avoid excessive agitation.

IMPORTANT: ONCE AMPULE IS BROKEN, USE REAGENT WITHIN 24 H. EACH AMPULE PROVIDES SUFFICIENT REAGENTS FOR 96 SAMPLES.

Sample preparation

Please follow validated DNA extraction instrument and reagents .

The following DNA extraction protocols were tested successfully with The ***DeepChek® Assay 13-Plex KB Drug Susceptibility Testing (V1.x) (RUO)***.

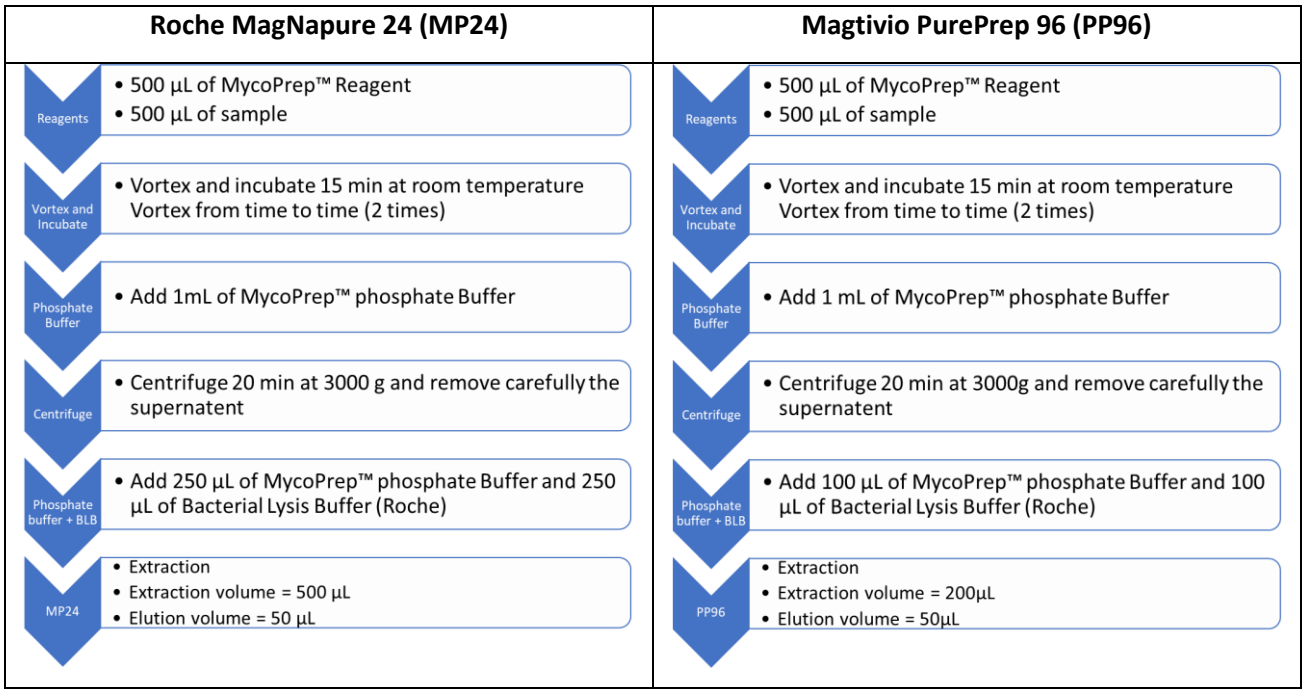

Figure 3: sample preparation and DNA isolation steps before the PCR using DeepChek® Assay 13-Plex KB Drug Susceptibility Testing (V1.x)

DNA extraction

To achieve optimal and sensitive DNA analysis, it is recommended to start from decontaminated and concentrated sputum sediments and to elute in the minimum volume (i.e., from fresh samples, to be eluted in 50µL), required for your laboratory validated extraction kit (MagNA Pure Nucleic Acid Isolation Kit I (Roche Diagnostics) or MagSi-NA Pathogens kit (Magtivio)).
Note: For MagNA Pure 24 instrument and reagents (Roche), you can perform DNA extraction for 24 samples, whereas with PurePrep 96 instrument and reagents (Magtivio), the DNA extraction is performed for 96 samples.
Note: please refer to relevant manufacturer's Instructions for Use (IFU) to proceed with the instrument and related reagents protocols.

Multiplex PCR

Please proceed with the following steps for an effective PCR using the ***DeepChek® Assay 13-Plex KB Drug Susceptibility Testing (V1.x) (RUO)*** (REF 128A24).

1. Thaw extracted DNA template, primer mix, the master mix and place them on ice. Load all the tubes into the centrifuge. Spin the samples at 11000 g during 10 seconds. And, then aspirate and discharge the solution several times before the dispensing.
2. Prepare a premix according to next table. The premix typically contains all the components required for PCR except the DNA template. Prepare a volume of premix greater (n+1) than that required for the total number of reactions to be performed. Premix needs to be made fresh with each batch of samples.

	Multiplex PCR (µL) for 1 sample	**Multiplex PCR (µL) for 24 samples + 1 negative control**
Master Mix	25.0 µL	625.0 µL
Primer Mix	13.0 µL	325.0 µL
Total volume	38. 0 µL	950.0 µL

Table 6: Premix components for a run of 24 samples to proceed with the multiplex PCR

3. Dispense 38 µL into each PCR tube. Mix by pipetting up and down a few times.
4. Add 12 µL of DNA template to each PCR tube. Mix by pipetting up and down a few times.
5. A total volume of 50 µL is required for each sample.
6. Program the thermal cycler with the following cycling program.

	Time	**Temperature**	**Cycle**
DNA pol Activation	5 min	95 °C	1
PCR	15 sec	95°C	35
	30 sec	65 °C	
	1min 30 sec	72°C	
Final elongation	10 min	72°C	1
Cooling	∞	4°C	1

Table 7: PCR cycling program

7. [Recommended] The PCR products can be controlled through electrophoresis on an agarose gel. Check the intensity of the signal. Run 5 µl of undiluted PCR product on an agarose 2%.

Note: the following figure shows example of PCR products successfully sequenced on an Illimuna iSeq100 system. If your specimens do not show bands on the revelation using the Agarose Gel; perform another extraction and run again the PCR reaction.

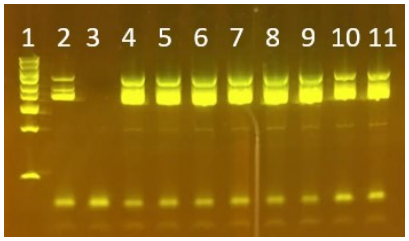

Figure 4: Revelation of the Multiplex PCR products on Agarose 2%. 1 : DNA Ladder. 2 : positive control. 3 : Negative control. 4 to 11 : Positive samples

RT-PCR troubleshooting guide

Check the concentration, storage conditions and quality of the starting template. For optimal results use fresh/frozen specimen and proceed with a fresh DNA extraction.

PCR products purification

Before sequencing, first make sure your PCR products have been purified.

Next Generation Sequencing

After the amplicon verification, the specimens are ready for the NGS kit processing.

Illumina

- **116B24 / 116B48 / 116B96 | ABL DeepChek® NGS LIBRARY PREPARATION V2 (24/48/96 reactions).**
- **124B24 / 124B48 / 124B96|ABL DeepChek® Adapters V2 (24 / 48 / 96).**
- **MS-103-1003 |** MiSeq Reagent Nano Kit, v2 (500 cycles) or
- **FC-420-1003 |** Mid Output kit Reagents (2x150) or
- **20021533 |** iSeq 100 i1 Reagent (2x150) or
- **20024908 |** NextSeq 500/550 High Output Kit v2.5 (300 Cycles).

User shall then follow the Denaturation and Dilution of the Libraries Guide and instructions for use from the manufacturer.

Ion Torrent

- **4471269** | Ion Xpress™ Plus Fragment Library Kit
- **4471250** | Ion Xpress™ Barcode Adapters 1-16 Kit
- **4484355** | Ion 318™ Chip Kit v2.

User shall then follow the instructions for use from the manufacturer.

NGS data analysis

NGS files containing nucleotide sequences are analyzed by a downstream analysis software (i.e., the ***BacterioChek®*** software which is the TB module (#TM) of the ***DeepChek® Software*** (ABL, #S-12-023)).

Users shall then follow the software user guide.

Product quality control

In accordance with ABL's Quality Management System, each lot of the assay is tested against predetermined specifications to ensure consistent product quality. Certificates of Analysis are available upon request.

Symbols

 <N>	Contains reagents enough for <N> reactions		Consult instructions for use
	Caution		Negative control
	Catalog number		Positive control
	Use by		Temperature limitation
	Manufacturer		Serial Number
	Country and date of manufacturing	**R*n***	R is for revision of the Instructions for Use (IFU) and n is the revision number
	Distributor		

Contact Information

For technical assistance and more information, please see our Technical Support Center at Online: https://ablsa.odoo.com/fr_FR/issue; Email: support-diag@ablsa.com; Call +339 7017 0300 Or contact your ABL Field-Application Specialist or your local distributor. For up-to-date licensing information or product-specific disclaimers, see the respective ABL Assay User Guide. ABL User Guides are available at **www.ablsa.com/ifu** or can be requested from ABL Technical Services or your local distributor.

Manufacturer and distributors

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