INSTRUCTIONS FOR USE

ABility STIplex Resist+

Real-time PCR *C. trachomatis*, *N. gonorrhoeae*, M. *genitalium* (MG) and MG macrolides resistance multiplex detection kit

For investigation use only (IUO) - Not for use in diagnostic procedures.

1. LIST OF PRODUCTS

Product	Commercial reference	Number of reactions
PCR kit	AB0027IUO	Σ 96 (96 possible reactions, including controls)

2. INTENDED USE

Clinical conditions	Sexually transmitted infections (STIs) have become a public health priority due to their increasing prevalence and the worrying emergence of antibiotic resistance, particularly to macrolides, which are commonly used to treat infections caused by *Mycoplasma genitalium.*
Intended use	ABility STIplex Resist+ is an *in vitro* nucleic acid amplification test for the qualitative detection of DNA from *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and *Mycoplasma genitalium* (MG), as well as mutations associated with macrolide resistance (MR) in MG. The kit can be used with the following sample types:urine, vaginal swabs in women and anal swabs, from symptomatic and asymptomatic patients. This kit can be used as an aid in the clinical diagnosis of CT, NG and MG infections. **For use in performance studies only, not for the diagnosis of patients.**
Test principle	ABility STIplex Resist+ is a qualitative multiplex *in vitro* diagnostic test using real-time PCR. The kit contains oligonucleotides (primers and TaqMan™ probes) that specifically detect: - the presence of *C. trachomatis* (*ORF6* and *ompA* genes*).* - the presence of *N. gonorrhoeae (porA* and *opa* genes*)* - the presence of *M. genitalium (mgpA* gene *and* 23S WT ribosomal RNA gene) - possible macrolide resistance of *M. genitalium* (A2058G, A2059G, A2058C, A2059C and A2058T mutations in the 23S ribosomal RNA gene). The process involves nucleic acid extraction, followed by PCR amplification and real-time detection.

Target population	Asymptomatic and symptomatic men and women for the detection of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG) and *Mycoplasma genitalium* (MG), as well as for screening for macrolide resistance in MG.
Intended users	Qualified laboratory personnel trained in good laboratory diagnostic practice and molecular biology techniques, in particular real-time PCR.
Environment of use	Medical biology laboratories with a structure that complies with national health authority recommendations.
Type of sample required	Clinician-collected and self-collected vaginal swabs, anal swabs and urine samples.
Manual or automated steps	- Automatic DNA extraction - Manual or automatic pipetting of samples and reagents - Manual loading of the thermal cycler - Automated real-time PCR and fluorescence detection - Manual interpretation of results
Type of analysis	Qualitative

3. TECHNICAL SPECIFICATIONS

<table>
<tr><td>Sampling/transport medium</td><td>• UTM universal transport medium®
• Amies medium</td></tr>
<tr><td>Recommended extraction methods</td><td>• DNA extraction using magnetic beads :
Nucleic Acid Extraction Kit (Ref. 1000021043/1000021042, Wuhan MGI Tech Co., Ltd)</td></tr>
<tr><td>Recommended instruments and accessories</td><td>
<table>
<tr><th>Instrument name</th><th>Manufacturer</th></tr>
<tr><td>Extractor and dispenser :
Smart GUARD</td><td>Appolon Bioteck</td></tr>
<tr><td>Thermocycler :
CFX Opus 96 Dx Real-Time PCR, combined with Bio-Rad CFX Maestro Dx SE software</td><td>Bio-Rad</td></tr>
</table>
</td></tr>
<tr><td>Target genes</td><td>- cryptic plasmid ORF6 and ompA (Chlamydia trachomatis)
- opa and porA (Neisseria gonorrhoeae)
- mgpA and 23S ribosomal RNA WT (Mycoplasma genitalium)
- Mycoplasma genitalium 23S rRNA mutations (A2058G, A2058C, A2058T, A2059G and A2059C, (Escherichia coli numbering)
- GAPDH (human genomic DNA)</td></tr>
<tr><td>Fluorescence channels</td><td>- FAM (Chlamydia trachomatis)
- ROX (Neisseria gonorrhoeae)
- Cy5.5 (Mycoplasma genitalium)
- HEX (Mycoplasma genitalium 23S rRNA mutation)
- Cy5 (human genomic DNA)</td></tr>
</table>

4. KIT CONTENTS

- **List of kit reagents :**

Reagents	Commercial reference	Part Number « P/N »	Colour of cap	Contents	Quantity	Volume
Mix	AB0027IUO	STIR+Mix	Transparent	Reaction mix : - Specific primers and probes for the detection of : • *C. trachomatis,* • *N. gonorrhoeae,* • *M. genitalium,* • *M. genitalium* macrolide resistance markers • human genomic DNA. - Master Mix	1 tube	1.5 mL
Positive control	AB0027IUO	STIR+CP	Red	Synthetic DNA corresponding to : • *C. trachomatis,* • *N. gonorrhoeae,* • *M. genitalium,* • macrolide resistance markers for *M. genitalium* • human GAPDH gene in TE buffer	1 tube	120 µL
Negative control	AB0027IUO	STIR+CN	Blue	Synthetic DNA corresponding to the human GAPDH gene in TE buffer	1 tube	120 µL

Description of reagents and limitations
The substances contained in this kit are not classified as dangerous substances under Regulation (EC) No 1272/2008.

5. TRANSPORT, STORAGE AND STABILITY OF THE DEVICE

Steps	Details of conditions
Transport	- The kit must be transported between -15°C and -80°C (dry ice).
Storage/Preservation	- Reagents should be stored away from direct sunlight and humidity. - Storage temperatures should be between -25°C and -15°C. - Unopened shelf life: refer to the expiry date on the product label.
Shelf life after first opening of tubes **In-use stability**	- The mix should be used away from direct sunlight. - Do not leave the kit at room temperature (between +15°C and +25°C) for more than 24 hours. Keep the tubes protected from light during this period. - Do not use after the expiry date.

6. EQUIPMENT AND ACCESSORIES REQUIRED BUT NOT SUPPLIED WITH THE DEVICE

Step	Type of equipment
Laboratory equipment	Freezer at -20°C, refrigerator at +4°C Powder-free disposable gloves Laboratory coat Benchtop centrifuge for 1.5 ml and 2.0 ml tubes and/or strips Centrifuge for PCR plates Benchtop vortexer Adjustable micropipettes P10, P20 and P200 Sterile filter cones for 10 µL, 20 µL and 200 µL Microtubes, strips or opaque 96-well 0.2 mL PCR plates compatible with the CFX Opus 96 Dx thermal cycler (Bio-Rad) Specific optical films for PCR (or caps if strips are used) Microbiological Safety Cabinet (MSC) for handling class I or II pathogens Cold rack or crushed ice For nucleic acid extraction and real-time PCR, please refer to section 3 - technical specifications.
Extraction	Smart GUARD (Appolon Bioteck
PCR Amplification	CFX Opus 96 Dx Real-Time PCR with Bio-Rad CFX Maestro Dx SE software
Other reagents	Molecular biology quality water DNA-RNA decontamination products

7. SAMPLE COLLECTION PROCEDURES

Samples for DNA extraction must be collected and transported in accordance with professional guidelines and processed by the laboratory as quicklly as possible.

Refer to the recommendations of the supplier of the selected collection device.

8. USE PROTOCOL

8.1 NUCLEIC ACID EXTRACTION

- DNA extraction using magnetic beads: please refer to the instructions for use of the *Nucleic* Acid *Extraction Kit* (Ref. 1000021043/1000021042, Wuhan MGI Tech Co., Ltd) and the Smart GUARD automated magnetic bead extraction system (AB0096, Appolon Bioteck).

  When using the Smart GUARD, please ensure that the volume of sample available is greater than 350 µL (200 µL taken by the automated system). At the end of the extraction process, a volume of approximately 35 µL of nucleic acid solution is obtained. The time required for the extraction step is approximately 80 minutes.

  Note: No extraction is required for controls; they are ready for PCR use.

- Quality control of the purity and quantity of DNA after extraction: follow the recommendations for the extraction method.
  The following parameters are recommended for the spectrophotometer: A_{260}/A_{280} (ratio of absorbance at 260 nm to absorbance at 280 nm) between 1.7 and 1.9. The minimum concentration required for the PCR reaction is 10 ng/µL.

8.2 REAL-TIME AMPLIFICATION

Distribution of reagents in the PCR plate can be carried out by the Smart GUARD automated system following nucleic acid extraction, or manually.

a) Using the Smart GUARD

In the case of automatic dispensing with Smart GUARD, molecular biology grade water can be used as NTC (No Template Control).

For the distribution of reagents in the PCR plate:

- Follow the Smart GUARD instructions for use, ensuring that you use the appropriate script for the extraction kit you are using.
- In the Smart GUARD control software, enter the volume of mix per reaction (15 µL) and the volume of sample extracted per reaction (5 µL).

Please note: if you wish to re-use the positive and negative control tubes, remember to keep them when you have finished using the Smart GUARD.

b) Manual preparation

1. Thaw the reagents on a cold block for 15-25 minutes, depending on the environment.
2. Draw up a detailed plate layout including patient codes and controls, preferably using CFX Maestro Dx SE software.
3. For each real-time PCR experiment, plan the number of reactions as follows:
    - 1 reaction for each sample (patient) to be tested
    - 1 reaction for the positive control
    - 1 reaction for the negative control
    - 1 negative PCR control reaction without DNA (molecular biology quality water) (NTC = No Template Control). The use of this control is strongly recommended.
4. Use a cold block to dispense the mix and samples.
5. Briefly vortex all the tubes in the kit and homogenise the samples.
6. Centrifuge all the tubes in the kit until the liquid has sunk to the bottom of the tube.
7. Dispense the reaction mixes according to **Table 1**:
    a. Dispense 15 µL of the amplification mix into each PCR well according to the number of patients and controls.
    b. Add 5 µL of each extracted DNA according to the plate plan.
    c. Place 5 µL of the positive control in the corresponding control well.
    d. Add 5 µL of negative control to the corresponding control well.
    e. Add 5 µL of NTC according to the plate plan.
    f. Seal the plate with optical adhesive film.
    g. Briefly centrifuge the PCR plate to remove any air bubbles that may form at the bottom of the wells and interfere with the reading of the fluorescent signal.
    h. Place the plate in the thermal cycler, making sure that it is facing the right way up.
    i. Finalise the thermal cycler settings:
        - *Configure the thermal amplification profile in terms of number of cycles, temperature and duration (***Table 2***).*
        - *Set the reaction volume ("Sample Volume"): 20 µL*
        - *Select detection channels: FAM, HEX, ROX, Cy5 and Cy5.5*
        - *Any other parameters*
    j. Start the PCR .

Table 1: VOLUMES TO BE DISTRIBUTED PER PCR WELL

Product	Volume/ well Sample	Volume / well Positive control	Volume / well Negative control	Volume / well NTC (if necessary)
ABility Mix STIplex Resist+	15 µL	15 µL	15 µL	15 µL
Sample DNA extract	5 µL	-	-	-
Positive control	-	5 µL	-	-
Negative control	-	-	5 µL	-
NTC (molecular biology quality water)	-	-	-	5 µL
Total reaction volume	**20 µL**	**20 µL**	**20 µL**	**20 µL**

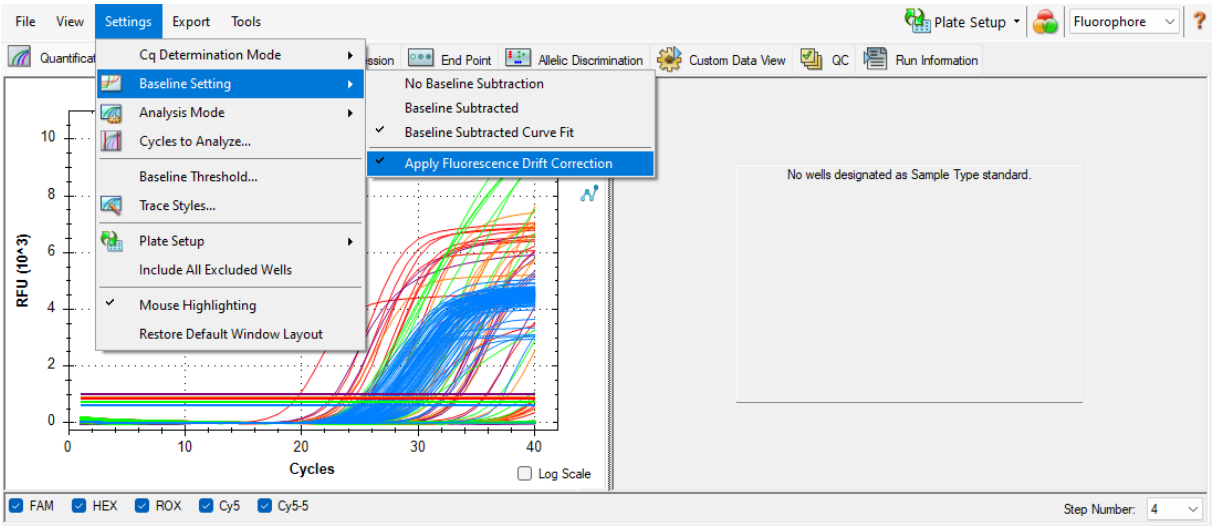
Table 2: THERMAL AMPLIFICATION PROFILE

Steps	Process	Programme		Cycles	Acquisition of fluorescence
		Duration	Temperature (°C)		
1	*Taq* polymerase activation	30 s	95°C	1	Not applicable
2	DNA denaturation	10 s	95°C	45	Not applicable
3	Amplification	15 s	60°C		FAM HEX ROX Cy5 Cy5.5

9. ANALYSIS OF RESULTS

At the end of the run, configure the following parameters in the CFX Maestro Dx SE thermal cycler software:

1. Select the Quantification tab.
2. Select Settings > Baseline Setting > Baseline Substracted Curve Fit and Apply Fluorescence Drift Correction.



3. Check the default fluorescence threshold lines and adjust them if necessary. To do this, select the wells containing the positive and negative controls on the plate plan to display their amplification curves.
4. A result is considered POSITIVE (+) if the reaction generates an amplification curve crossing the fluorescence threshold line at a threshold cycle (C_T) ≤ at 45.

The interpretation of the results is detailed in **Table 4**.

9.1 QUALITY CONTROL

The expected results for the controls are shown in **Table 3**.

Table 3: QUALITY CONTROL

	Channel (target)					
	FAM (CT)	**ROX (NG)**	**HEX (MG-SNP)**	**Cy5.5 (MG)**	**Cy5 (hGAPDH)**	**Interpretation**
Positive control	+	+	+	+	+	Valid result
Negative control	-	-	-	-	+	Valid result
NTC	-	-	-	-	-	Valid result

If the results do not conform to the table, repeat the amplification steps.
If the controls and NTC are correct, the PCR results are considered valid.

9.2 INTERPRETATION OF RESULTS

Table 4: INTERPRETATION OF RESULTS

FAM (CT)	**ROX** (NG)	**Cy5.5** (MG)	**HEX** (MG-SNP)	**Cy5** (hGAPDH)	**Interpretation of results**
+	-	-	-	+/-	POSITIVE for *C. trachomatis*
-	+	-	-	+/-	POSITIVE for *N. gonorrhoeae*
-	-	+	-	+/-	POSITIVE for *M. genitalium* 23S rRNA mutation not detected ➔ Wild type
-	-	+	+	+/-	POSITIVE for *M. genitalium* 23S rRNA mutation detected ➔ Macrolide resistant strain
+	+	+	+/-	+/-	POSITIVE for *C. trachomatis*, *N. gonorrhoeae* and *M. genitalium* ➔ Co-infection *M. genitalium* can be detected with or without detection of macrolide resistance.
-	-	-	-	+	NEGATIVE The sample contains no DNA of interest.

FAM (CT)	ROX (NG)	Cy5.5 (MG)	HEX (MG-SNP)	Cy5 (hGAPDH)	Interpretation of results
-	-	-	-	-	INVALID

10. TROUBLESHOOTING

Problems	Possible causes	Solutions
Invalid results (sample and/or control)	Alteration of the mix	• Freezing/thawing cycles are not permitted. • Use a cold block when distributing the mix and samples. • Taq polymerase enzymes are sensitive to temperature variations. Do not leave the kit at room temperature for more than 24 hours. • Protect from light. • Check the expiry date of the batch used.
Invalid sample result	Failure to comply with sample collection, transport and storage conditions.	• Follow the instructions for prepation, transport and storage of samples. • Check the time between sample collection and PCR analysis.
Invalid sample result	Problem during nucleic acid extraction	• Check that samples are thoroughly homogenised before the extraction step. • Check all equipment and extraction solutions used to extract samples. • Repeat the extraction process. • Always carry out preventive maintenance on extraction equipment in accordance with the manufacturer's recommendations.
Incorrect control result or invalid results	Reagents or sample dispensing error	• Draw up a plate layout to limit the risk of dispensing errors. • Check the calibration of pipettes, which should be subject to regular metrological checks. • Ensure that the mix, controls and samples are well homogenised before distribution to the PCR plate wells.
Incorrect control result or Invalid results	Thermocycler programming or loading error	• Check that the thermal cycler used is compatible with the one validated with the kit. • Check all thermal cycler programming parameters (detection channel and mode, number of cycles, temperature, time, reaction volume, etc.). • Check that the plate is correctly oriented.
Invalid results	Amplification problem	• Check the adjustment of the threshold line. • Check the fluorescence intensity of the amplification curves. • Check the NTC and controls. • Check that the run validation criteria have been met as indicated in section §9.1 QUALITY CONTROL.

Problems	Possible causes	Solutions
		• Check that the thermal cycler used is the one validated with the kit. • Check the Peltier block's performance according to the manufacturer's recommendations. • Presence of PCR inhibitors. • Carry out preventive maintenance on PCR equipment in accordance with the manufacturer's recommendations. • Check that the plastic consumables used are those recommended for the instrument used (opaque semi-skirted plate, low profile tubes/plate, etc.). • Check that the PCR plate is properly sealed with the optical film.
Incorrect control result or Invalid results	Contamination during experimentation	• Decontaminate all small laboratory equipment with products suitable for removing nucleic acids. • Change the products used (water, PCR reagents, etc.). • Decontaminate the thermocycler according to the manufacturer's recommendations.
Incorrect NTC result	Contamination of the NTC	• Decontaminate all small laboratory equipment with products suitable for removing nucleic acids. • Decontaminate the thermocycler according to the manufacturer's recommendations. • Change the molecular biology-grade water used. • Repeat the analysis.
Incorrect results	False negatives	• Failure to follow sample collection procedures. • Failure to comply with sample transport or storage conditions. • Use of unauthorised or expired reagents. • Use of reagents not stored under the conditions specified in the user manual. • Failure to follow the kit instructions.
Incorrect results	False positives	• Cross-contamination between samples during sample handling. • Contamination by the positive control during handling.

11. PRECAUTIONS FOR USE AND LIMITATIONS

- *Precautions prior to use of the kit:*

This kit has been designed for use in performance investigations. Performance has not been established. This product is not intended for the diagnosis, prevention or treatment of any disease.

- Read the entire instructions carefully before starting to use the kit. Failure to follow the instructions in this user manual may lead to incorrect results.
- The kit must be used by personnel trained in good laboratory practice and competent in molecular biology.

- Please consult the Safety Data Sheet (SDS) on the company's website (the substances contained in this kit are not classified as dangerous substances under Regulation (EC) No 1272/2008).
- Dry ice is carbon dioxide (CO_2) in a solid state. This gas is colourless, odourless and heavier than air, which can lead to an accumulation on the ground in confined or poorly ventilated spaces, increasing the risk of asphyxiation. Ensure that dry ice is only handled in well-ventilated areas to prevent the accumulation of CO_2.
- The integrity of the packaging and the device must be checked before use (no leaks/breakage, adequate number of tubes, correct volumes, etc.). Do not use the product if any damage is visible.
- Do not use the device if there are any signs of contamination (presence of foreign bodies in the tubes).
- Do not use kit reagents after the expiry date indicated.
- Compliance with sample collection procedures (collection, transport, storage) is necessary for optimal performance of this test, in order to avoid erroneous results.
- Do not use this kit with any sample other than that recommended in this instruction, as this may lead to incorrect results.
- Do not use the kit with any equipment other than that recommended in this instruction, as this may lead to incorrect results.
- Nucleic acid extraction and amplification must be performed using the equipment and methods listed in the instructions. For any other equipment not mentioned in the instructions, its effectiveness must be assessed by an internal laboratory validation procedure.
- For equipment not supplied but to be used in combination with the device, please refer to the device's instructions for use before use.

- *Precautions during use the kit:*
- Caution: Protect the mix from exposure to direct sunlight.
- Wear appropriate personal protective equipment (PPE), including a lab coat, gloves and eye protection, during all stages of handling the kit, to ensure user safety and prevent contamination.
- Samples must be considered potentially infectious. They should be prepared under a type II microbiological safety cabinet (MSC).
- Use separate workstations for sample preparation / nucleic acid extraction and amplification reactions. Never introduce an amplified product into the reagent preparation and/or nucleic acid extraction (sample preparation) area.
- Avoid microbial contamination of reagents during handling.
- Use only RNase- and DNase-free filter tips.
- Use new tips for each pipetting step.
- Close the kit component tubes immediately after use and never mix up the caps.
- Do not mix reagents from different batches or from the same batch in a single tube. Do not substitute reagents between batches.
- Avoid any contact between the kit reagents and the skin. In case of skin contact, wash immediately and thoroughly with water (see Safety Data Sheet).

- *Precautions after use of the kit/Limitations:*
- In case of an invalid result, please repeat the procedure.

12. CONTRAINDICATIONS

To date, APPOLON BIOTECK has not identified any contraindications for this product.

13. DISPOSAL OF THE DEVICE AFTER USE

To dispose of this device, refer to the national and/or international regulations in force, applicable to the disposal of hazardous, biological or infectious waste.
Unused reagents from the kit can be considered non-hazardous and disposed of according to standard laboratory procedures.

14. DISCLAIMER

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APPOLON BIOTECK reserves the right to modify its products and services at any time. This instructions for use is subject to change without notice. APPOLON BIOTECK accepts no liability for errors or omissions or for any damages arising from the application or use of this information.

15. SYMBOLS AND LOGOS USED

Symbol	Indication	Symbol	Indication
	Manufacturer		Catalogue reference
	Batch number		Use by date
 *n*	Sufficient content for "n" reactions		For investigation use only (IUO) - Not for use in diagnostic procedures
	Consult instructions for use or consult electronic instructions for use	P/N	Part number, component identification
	Storage temperature limits		Caution
	Do not use if package is damaged and consult instructions for use		Keep away from sunlight
	Keep dry		Single use
X/2	Configuration of the medical device in two numbered boxes X/2	MIX	Tube containing the mix solution
CONTROL	Control	CONTROL +	Positive control

Symbol	Indication	Symbol	Indication
	Negative control		