AGREED BY:
Andrey Toropovsky
CEO

INSTRUCTION
FOR USE OF

	Generic Device Term	Commercial name	Catalogue reference number
1	Kit of reagents	Kit for fetal SRY gene detection in maternal blood "Test-SRY" for 50 tests	SRY50
2	Kit of reagents	Kit for fetal SRY gene detection in maternal blood "Test-SRY" for 100 tests	SRY100
3	Kit of reagents	Kit for fetal SRY gene detection in maternal blood "Test-SRY Plus" for 50 tests	SRY50+
4	Kit of reagents	Kit for fetal SRY gene detection in maternal blood "Test-SRY Plus" for 100 tests	SRY100+

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INTENDED USE

Reagents Kits "Test-SRY Plus" and "Test-SRY" are intended for fetal *SRY* gene location in pregnant woman blood by polymerase chain reaction (PCR) with hybridization-fluorescence detection. Material for PCR is DNA isolated from blood plasma.

Application area – laboratory diagnostics for detection of DNA and sex-determining gene (SRY) in blood plasma of pregnant women. The kit may be used to determine the minimal gene concentrations and other biological fluids. The kit is intended for use in medical and healthcare institutions including diagnostic and criminalistics laboratory as well as for scientific purpose.

Currently it's known about over 300 genes localized in X chromosome which caused hereditary diseases. These are genes of X-linked ichthyosis, fragile X syndrome, Coffin-Lowry syndrome, Payne syndrome, Opitz syndrome and many other diseases. Moreover intrauterine fetus sex determination is possible using USI-diagnostics although this method is applicable from 22nd week of pregnancy and it's considered that its accuracy doesn't prevail 70%.

Use of diagnostic kits for fetal sex determining region Y (SRY) gene detection in maternal blood from 10^{th} embryologic week allows specialist determining fetal gender.

KIT CHARACTERISTICS

Kit composition

Reagents kit «Test-SRY» **includes:**

Reagent	Volume, ml		Tube number	
	50 tests	100 tests	50 tests	100 tests
SRY PCR Mix	1,200	2,400	1	2
GAPDH PCR Mix	0,480	0,960	1	2
Taq-polymerase	0,085	0,170	1	2
Male control DNA (Positive Control, PC)	0,960	1,920	1	2
Deionized water (No Template Control, NTC)	1,920	3,840	2	3

Reagents kit «Test -SRY **Plus**» **includes:**

Reagent	Volume, ml		Tube number	
	50 tests	100 tests	50 tests	100 tests
PCR Buffer	1,680	3,360	2	3
SRY primer Mix	1,200	2,400	1	2
GAPDH primer Mix	0,480	0,960	1	2
Male control DNA (Positive Control, PC)	0,720	1,440	1	2
Deionized water (No Template Control, NTC)	1,440	2,880	1	2

Number of tested probes

Kits are supplied in two variants – for 50 (420 reactions) and 100 tests (840 reactions) including all positive and negative controls. Every kit has 20% reagents for compensation of loss at pipetting. That is there is enough reagents for 50 and 100 clinical samples even in the case of single tests with all reagent controls in the kit of variant "50" and "100" correspondingly. Parallel test of several clinical samples is possible. In this case, up to 100 and 200 clinical samples can be tested using in the kit of variant "50" and "100" correspondingly as reagent consumption for positive and negative controls decreases at parallel testing.

PRINCIPLE OF PROCEDURE

DNA SRY gene detection by polymerase chain reaction (PCR) with hybridization-fluorescence detection includes three stages:

1) DNA extraction from test material;

2) DNA PCR-amplification with parallel hybridization-fluorescence detection which is conducted during PCR.

3) Results interpretation.

Cell-free fetal DNA extraction from test material is performed using recommended procedure of extraction. Then amplification reactions of *SRY* and *GAPDH* genes are performed with obtained DNA probes in reaction buffer using primers specific to these DNA focuses and enzyme Taq-polymerase. Reaction mixture for amplification consists of fluorescent-labelled oligonucleotide probes which are hybridized with complementary focus of amplified DNA-target and destructed by Taq-polymerase and as a result increase of fluorescence intensity occurs. This allows registering of specific amplification product accumulation by measuring of fluorescent signal intensity. Fluorescent signal detection is performed during PCR using cycler with fluorescent signal detection system in real-time mode. Product of DNA amplification of *SRY* gene and *GAPDH* gene is detected by channel corresponded to fluorophore FAM.

ANALYTICAL CHARACTERISTICS

Analytical sensitivity – 10 genome equivalents/ µl (GE/µl).

Analytical specificity of *SRY* gene detection – 100%.

Range of determined concentrations of *SRY* gene is from 10 to 10000 GE/µl.

Kit is intended for qualitative determination of *SRY* gene.

There must be no interfering substances during *SRY* gene determination when using recommended methods of DNA extraction.

PRECAUTIONS FOR HANDLING

Agents of chemical and biological danger are absent in the kit.

The work should be conducted in the laboratory performed molecular-biological (PCR) tests of clinical material. The following requirements should be always met during work:

- Dispose carefully unused reagents carefully in accordance with local regulations.

ATTENTION! *Opening of tubes and content spraying are unacceptable during waste disposal* after amplification as this can lead to contamination of laboratory area, equipment and reagents by PCR products.

- Use the kit strictly for purpose intended in accordance with this instruction.
- Only specially trained staff should work with the kit.
- Do not use the kit after its expiry date.
- Avoid contact with skin, eye, and mucous membrane. Immediately flush the affected area with water and seek medical attention.

EQUIPMENT AND MATERIALS

1. PCR-box (for example, BAV-PCR-Laminar-C, Laminar systems, Russia).
2. Vortex (for example, TETA-2, Biocom, Russia).
3. Kit of electronic and automatic adjustable (for example, Lenpipet, Russia).

4. Disposable tips with aerosol barrier up to 200 µl, up to 100 µl, up to 20 µl, and up to 10 µl (for example, Axygen, USA).

5. Tip racks (for example, Axygen, USA) and microtubes 0.5 (0.2) ml holder (for example, «InterLabService», Russia).

6. Refrigerator of 2 to 8°C with freezer below -16°C.

7. Individual coat and disposal gloves.

8. Container with cap for disinfecting solution.

9. Cycler of rotary tipe example, Rotor-Gene 3000 or 6000 (Corbett Research, Australia) or plate amplifier, for example, IQ5, (BioRad, USA), Mx3000P (Stratagene, USA) or equivalents.

10. Disposable polypropylene tubes for PCR:

а) by 0.2 ml (flat cap, non-stripped), (for example, Axygen, USA) for set in rotor by 36 tubes – for PCR apparatus in real time with detection through the tube bottom (for example, Rotor-Gene).

б) by 0.2 ml (dome-shaped cap) (for example, Axygen, USA) – for PCR apparatus in real time with detection through the cap (for example, IQ5, Mx3000P).

TEST SAMPLES

For test conduction the peripheral blood plasma is used. Accepted anticoagulants: EDTA, CFDA.

When using CPDA tubes as anticoagulant it is allowed to transport whole blood during 2 days without freezing. When using EDTA tubes, plasma should be separated during 2-3 hours since blood drawing.

Blood (at least 8-10 ml) is drawn in the tube with EDTA anticoagulant for plasma obtaining. Closed tube with blood is turned over several times. The tube with blood is centrifuged for 15 min at 2000-3000 g. Then upper layer of plasma is carefully withdrawn and transfer it to the separate disposable tube avoiding penetration of leukocytes clots and layers with erythrocytes in taken material. Plasma is centrifuged for 15 minutes at 13000 g or for 10 minutes at 16000 g and upper layer is withdrawn again in the separate tube without touch of sediment in the tube bottom.

Obtained plasma can be used for DNA extraction or frozen at not more than -70°C for further use. DNA should be extracted from at least 1 ml of plasma (2 ml is recommended) and dissolved in final volume of 60-80 µl.

If necessary, it is allowed to transport the obtained plasma at a temperature from 4 ° C to 8 ° C for no more than five days in a thermal container with ice pack. Upon receipt of the material immediately proceed to the procedure of DNA extraction.

After completion of DNA isolation, start the PCR-reaction. Fetal DNA is present in the mother's blood in low concentrations and damaged state. It may be destroyed during storage that can lead to false-negative results.

TESTING PROCEDURE

PCR-testing includes the following stages:

- DNA extraction from test samples;
- Amplification with fluorescence detection of amplification products in the «real-time» mode;
- Interpretation of results.

DNA EXTRACTION FROM TEST SAMPLES

For DNA extraction, the following reagent kits are recommended:

- Kit for DNA isolation from blood plasma «DNA-Plasma-M» (TestGene, Russia),
- Kit for DNA isolation from 2 ml of blood plasma DNA-plasma-2 (TestGene, Russia)
- Kit for DNA isolation from 1 ml of blood plasma DNA-plasma-1 (TestGene, Russia)
- Kit for free-circulating DNA isolation from blood plasma «DNA-Plasma-M-RT» (TestGene, Russia)
- Kit for DNA isolation from clinical material DNA-sorb-B (Amplisens, Russia)
- NucleoSpin® Blood (MACHEREY-NAGEL, Germany)
- QIAamp Circulating Nucleic Acid Kit, QIAamp UltraSens Virus Kit (QIAamp, Germany)

or equivalents intended to extraction of circulated nucleic acids from biological fluids.

AMPLIFICATION WITH DETECTION IN REAL TIME

Total test volume – 20 µl.

ATTENTION! Test volume should not be changed. Method sensitivity is strongly decreased at volume change!!!

ATTENTION! When working with DNA it's necessary to use only disposable sterile plastic materials with special labeling "DNase-free".

A. Preparation of tubes for amplification

Selection of tubes for amplification depends from used cycler. Disposable tips with filters are used for reagents, DNA probes and control samples entry to tubes.

ATTENTION! Components of reaction mixture should be mixed just before the test. Reagents should be mixed for required tests number including testing of test and control samples in accordance with design (calculation) tables.

1. Defrost all kit reagents (necessary at the current moment) before the work start and all drops should be sediment from tubes caps. Take Taq-polymerase (the kit «Test-SRY») from refrigerator before application avoiding long-term storage at room temperature.

2. After defrosting precipitate drops from tube caps by short centrifugation. Thoroughly mix the content of tubes at vortex in a few seconds, and precipitate drops from tube caps by short centrifugation.

3. Prepare the required tube number for amplification of test and control DNA samples. Select types of tubes, strips and plates depending from used apparatus.

4. When working with «Test-SRY» for preparation of reaction mixture mix all components in separate sterile tubes based on one reaction. It is necessary to use separate tip with aerosol barrier for every reaction component of each test.

5. When working with small volumes of thick fluids (such as Taq-polymerase) it's recommended to prepare the mix for 5-10 tests in order to select with automatic pipette not less than 1 μl of fluid. When selecting Taq-polymerase, it is not recommended to put the tip into reagent avoiding withdrawing of excess enzyme volume due to its transfer to external tip surface.

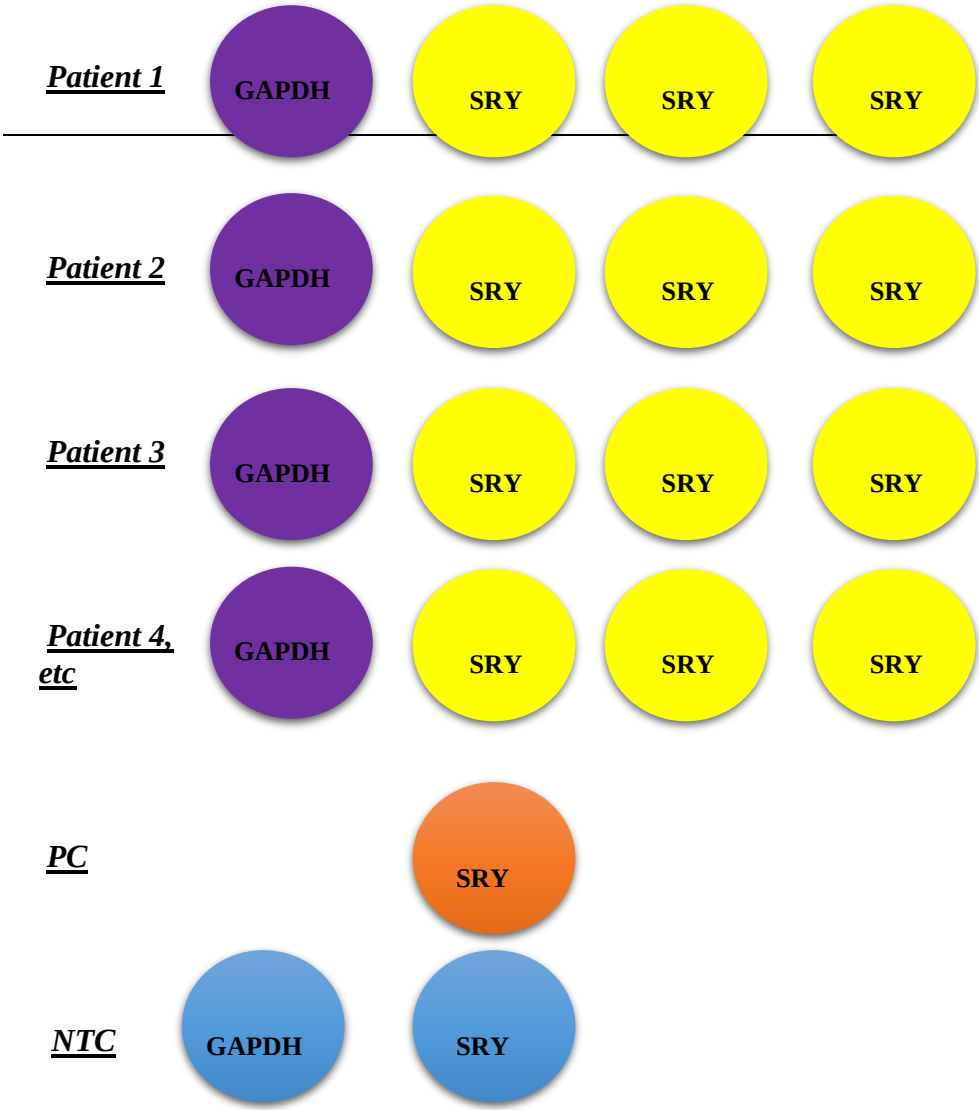
Probe Preparation

«Test-SRY»	
Tube 1 (SRY-1)	**Volume, µl**
Extracted DNA	15.8
Mix SRY PCR Mix	4.0
Taq-polymerase	0.2
Tube 2 (SRY-2)	**Volume, µl**
Extracted DNA	15.8
SRY PCR Mix	4.0
Taq-polymerase	0.2
Tube 3 (SRY-3)	**Volume, µl**
Extracted DNA	15.8
SRY PCR Mix	4.0
Taq-polymerase	0.2
Tube 4 (GAPDH)	**Volume, µl**
Extracted DNA	15.8
GAPDH PCR Mix	4.0
Taq-polymerase	0.2
Tube 5 (SRY PC)	**Volume, µl**
Positive control	15.8
SRY PCR Mix	4.0
Taq-polymerase	0.2
Tube 6 (SRY NTC)	**Volume, µl**
Deionized water	15.8
Mixture for SRY PCR	4.0
Taq-polymerase	0.2
Tube 7 (GAPDH NTC)	**Volume, µl**
Deionized water	15.8
GAPDH PCR Mix	4.0
Taq-polymerase	0.2

«Test-SRY **Plus**»	
Tube 1 (SRY-1)	**Volume, µl**
Extracted DNA	12.0
SRY primers Mix	4.0
PCR Mix	4.0
Tube 2 (SRY-2)	**Volume, µl**
Extracted DNA	12.0
SRY primers Mix	4.0
Taq-polymerase	4.0
Tube 3 (SRY-3)	**Volume, µl**
Extracted DNA	12.0
SRY primers Mix	4.0
Taq-polymerase	4.0
Tube 4 (GAPDH)	**Volume, µl**
Extracted DNA	12.0
GAPDH PCR Mix	4.0
PCR Mix	4.0
Tube 5 (SRY PC)	**Volume, µl**
Positive control	12.0
SRY primers Mix	4.0
Mixture for PCR	4.0
Tube 6 (SRY NTC)	**Volume, µl**
Deionized water	12.0
SRY primers Mix	4.0
PCR Mix	4.0
Tube 7 (GAPDH NTC)	**Volume, µl**
Deionized water	12.0
GAPDH primers Mix	4.0
Mixture for PCR	4.0

PIPETTING SCHEME

The arrangement of the tubes



Procedure of pipetting
for "Test-SRY plus"

1. **PCR buffer** (pipette 4 µl of the buffer into the well bottom of each tube).
2. **SRY primer Mix and GAPDH primer Mix** (pipette 4 µl on the tube wall according to the scheme).
3. **Isolated DNA for testing** (pipette 12 µl on the tubes wall according to the scheme).
4. **Deionized water** (No Template Control, NTC) (pipette 12 µl on the tubes wall according to the scheme).
5. **Male control DNA (Positive Control, PC)** (pipette 12 µl on the tubes wallaccording to the scheme).

"Test-SRY".
Calculation table for the PCR mix with Taq-Polymerase preparation.

Amount of patients	**SRY** µl	**TaqP** µl
1	24	1,2
2	36	1,8
3	48	2,4
4	60	3
5	72	3,6
6	84	4,2
7	96	4,8
8	108	5,4
9	120	6
10	132	6,6

GAPDH µl	**TaqP** µl
12	0,6
16	0,8
20	1
24	1,2
28	1,4
32	1,6
36	1,8
40	2
44	2,2
48	2,4

Calculation table for the PCR mix with Taq-Polymerase preparation.

1. PCR mix preparation (pipette 4,2 µl of the buffer into the well bottom of tubes according to the scheme).

2. Isolated DNA (pipette 15,8 µl on the tubes wall according to the scheme).

3. Deionized water (No Template Control, NTC) (pipette 15,8 µl on tubes wall according to the scheme).

4. Male control DNA (Positive Control, PC) (pipette 15,8 µl on the tubes wall according to the scheme).

B. Amplification with detection in «real time» mode

1. Put tubes in reaction module.

2. Program the apparatus for performance of the relevant amplification program and detection of fluorescent signal in accordance with FAM/Green channel.

Amplification program

Stage	Temperature, °C	Time	Total cycles
1	95	5 min.	1
2	94	10 sec.	50
3	62 *	50 sec.	

3. Start the performance of amplification program with detection of fluorescent signal in stage 3.

4. Start results analyses after program completion.

RESULTS REGISTRATION

Results registration is conducted using PCR apparatus software with detection in «real time» mode. Analysis of accumulation curves of fluorescent signal by one channel is performed:

- by **FAM/Green** channel it is registered signal evident on *SRY* and *GAPDH* amplified products accumulation.

Results are interpreted based on presence (or absence) of fluorescence curve crossing with the threshold established on the relevant level that is evident on the presence (or absence) of threshold cycle value «Ct» in the relevant field of results table for this DNA probe.

INTERPRETATION OF REACTION RESULTS

Interpretation of results in test samples

Obtained results are interpreted based on data on fluorescent signal level compared with background by relevant channels for control samples and DNA probes extracted from test samples. Interpretation is performed automatically using apparatus software.

The principle of results interpretation is the following:

Accumulation curves of fluorescence signal are analyzed for all samples and genes on the optical channel FAM (Green). Method of the threshold line (Ct) is used when determining the values of threshold cycles. In this case, Threshold is set on the rate of curves transition to exponential growth phase.

Sufficient amount of isolated DNA is a confirmation of reaction progress of *GAPDH* gene for test sample. Its threshold cycle (Ct) must not exceed the threshold cycle of positive control sample (PC). For example, if the Ct value of PC is 29.5 cycles, the Ct value of *GAPDH* for test sample is no more than 29.5 cycles. In this case, quantity and quality of isolated DNA is evaluated as enough. Interpretation of results is the next stage.

- Testing result is invalid if signal by channel FAM in *GAPDH* tube is lower than established threshold value or outputs after threshold cycle of male control DNA (PC) for 2 or more cycles.

If result for the probe is invalid, PCR of the relevant test sample should be repeated starting from repeated DNA extraction from plasma.

- DNA of sex-determining gene is identified if signals by FAM channel in two or three *SRY* tubes for this probe are higher than established threshold value and signal by FAM channel in *GAPDH* tube is higher than established threshold value;

- DNA of sex-determining gene is not identified if signals by FAM channel in three *SRY* tubes for this probe are lower than established threshold value and signal by FAM channel in *GAPDH* tube is higher than established threshold value and outputs not later than male control DNA (PC).

- Result is doubtful, if for this sample signal by FAM channel in one of three SRY tubes are higher than established threshold value. Result is doubtful if for this sample the signal by FAM channel in one or two SRY tubes are higher than established threshold cycle but value of «Ct» threshold cycle for one of these tubes is more than 45.

If it is obtained doubtful result for the sample, it is necessary to repeat DNA isolation from more plasma and perform repeated PCR-

testing for this patient through 2 weeks since another blood collection. In case of doubtful result, samples are considered positive.

The kit is unsuitable for further use if the signal by FAM channel in SRY PC tube is lower than established threshold cycle, and this result is consistently reproduced.

Interpretation of results in control samples

PCR test result is considered reliable only if correct results are obtained for the negative controls of amplification and positive controls of amplification.

Analysis Result Assessment for Test Samples

Result by fluorescence level			**Result**	**Remarks**
FAM channel (SRY-1,2,3)	**FAM** channel (GAPDH)	**FAM** channel (PC SRY)		
SRY 3 of 3 **Higher** than threshold value	**Higher** than threshold value	**Higher** than threshold value	Sex-determining region Y DNA **is identified**	Sex-determining region Y DNA is identified in the test sample
SRY 2 of 3 **Higher** than threshold value	**Higher** than threshold value	**Higher** than threshold value	Sex-determining region Y DNA **is identified**	Sex-determining region Y DNA is identified in the test sample
SRY 3 of 3 **Lower** than threshold value	**Higher** than threshold value and outputs not later than 2 cycles from male control DNA (PC)	**Higher** than threshold value	Sex-determination region Y DNA **is not identified**	Sex-determination region Y DNA is not identified
SRY 1 or 2 **Higher** than threshold value, but for one of them value of «Ct» threshold cycle is more	**Higher** than threshold value	**Higher** than threshold value	Doubtful	Test sample requires repeated testing

than 45.				
SRY 1 of 3 **Higher** than threshold value	**Higher** than threshold value	**Higher** than threshold value	Doubtful	Test sample requires repeated testing
–	**Lower** than threshold value	–	Invalid	Test DNA requires repeated isolation and testing
–	–	**Lower** than threshold value	–	Errors were made at reaction conduction or kit is unusable

Results for controls of different PCR stages

Control	Stage	Signal by FAM channel	Result
NTC	PCR	**Lower** than threshold value	Successful PCR
		Higher than threshold value	Contamination in genome DNA or PCR products

STORAGE AND HANDLING CONDITIONS

Manufacturer guarantees stable work of kits at observation of storage conditions during shelf period.

Storage. Reagents kit should be stored at 2 °C to 8 °C in the manufacturer packing during all shelf life.

After packing opening kit components should be stored at 2 °C to 8 °C during all shelf life.

Kit stored with violation of storage conditions are not to be applied.

Transportation. Reagents kit should be transported by all kinds of transport at the covered vehicles in accordance with rules of transportation acting on the transport of this type.

Kit must be transported at temperature from 2 °C to 8 °C during all shelf life. Transportation at room temperature (15–25°C) is acceptable but no longer than 3 days.

Atmosphere pressure is not controlled because it does not influence the sample quality.

For ensuring of transportation conditions during all transportation period the kit is placed into reusable polyurethane-foam thermal container with ice pack for temporary storage and transportation. Type, volume, ice pack amount at transported kits and thermal container volume are selected depending on duration and transportation conditions.

During transportation it is permissible to freeze the reagents kit.

Kits transported with violations of temperature conditions are not to be used.

Shelf life. 12 months. The kit shall not be used after the expiry date.

Shelf life of opened kit components. 12 months if stored at 2 °C to 8 °C.

Shelf life of kit components ready for operation. 1 hour if stored in an ice bath and complied with conditions that prevent components drying and contamination by outside biological material.

Utilization.

In accordance with classification of medical waste the kits refer to class A (epidemiologically safe waste approached on structure to municipal solid waste). Unused reagents in accordance with p. 4.28 of SanPiN 2.1.7.2790-10 «Sanitary and epidemiologic requirements to the address with medical waste» are not subject to use gather in the one-time marked packaging of any color (except yellow and red). Residual tubes and materials are utilized in accordance with MU 287-113 (Methodological instructions for disinfection, presterilization purification and sterilization of medical devices).

WARRANTY OBLIGATIONS AND RECLAMATIONS

The manufacturer guarantees the conformity of kits to technical requirements under transportation, storage and operation conditions established by technical specification.

If there are any complaints regarding the quality, undesired events that may cause adverse event (incident), send the information to the address:

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