AmpliSens® SARS-CoV-2-IT reagent kit

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Consult instructions for use
IVD	*In vitro* diagnostic medical device	Σ	Contains sufficient for <n> tests
VER	Version		Use-by-date
	Manufacturer	EC REP	Authorized representative in the European Community
	Date of manufacture	C–	Negative control of extraction
	Temperature limit	NCA	Negative control of RT-IT
	Keep away from sunlight	PCE	Positive control of extraction

1. INTENDED USE

AmpliSens® SARS-CoV-2-IT reagent kit is an *in vitro* isothermal nucleic acid amplification test for qualitative detection of SARS-CoV-2 RNA in the biological material (nasopharyngeal and oropharyngeal swabs) using reverse transcription and isothermal amplification (RT-IT) with fluorescence detection of amplified products. The material for RT-IT is RNA samples extracted from biological material. The reagent kit is used for complex laboratory diagnosis of COVID-19.

Indications and contra-indications for use of the reagent kit

The reagent kit is used for investigation of biological material taken from the persons who arrived from the regions where COVID-19 cases are registered, persons who had contact with COVID-19 patients, without distinction of form and presence of manifestation, patients with acute respiratory infection with COVID-19 suspicion.

There are no contra-indications with the exception of cases when the material cannot be taken for medical reasons.

Negative result of investigation does not rule out the possibility of infection with SARS-CoV-2 coronavirus and can be obtained if the virus content in the sample is below the specified analytical sensitivity.

NOTE: The results of analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF DETECTION

Principle of testing is based on the RNA extraction from the samples of test material, simultaneous RNA reverse transcription and isothermal amplification of cDNA fragments of the detected microorganism with fluorescence detection of amplicons.

RNA reverse transcription with the revertase enzyme and isothermal amplification of cDNA fragments with the use of specific primers and Bst polymerase enzyme are performed with the RNA samples obtained at the extraction stage. In the real-time isothermal amplification, the specific product is detected with the use of fluorophore which binds to double-stranded amplified products. The real-time monitoring of fluorescence intensities during the real-time isothermal amplification allows the detection of accumulating product without re-opening the reaction tubes after the run.

The result of isothermal amplification is registered in the following fluorescence channel:

Table 1

Channel for fluorophore	FAM
cDNA-target	SARS-CoV-2 cDNA
Target gene	*ORF1ab*

3. CONTENT

AmpliSens® SARS-CoV-2-IT reagent kit is produced in 1 form:

IT kit variant 200, REF H-4121-10-CE

IT kit variant 200 includes:

Reagent	Description	Volume, ml	Quantity
IT-mix SARS-CoV-2	colorless clear liquid	1.0	1 tube
IT-mix-E	clear liquid from colorless to light yellow colour	1.0	1 tube
C+ IT-SARS-CoV-2*	colorless clear liquid	0.1	1 tube
C–*	colorless clear liquid	1.0	2 tubes

* must be used in the extraction procedure.

IT kit variant 200 is intended for 200 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

For sampling and pretreatment

- Transport medium for storage and transportation of respiratory swabs.
- Reagent for pretreatment of viscous fluids (sputum, aspirates).
- 0.9 % of sodium chloride (sterile saline solution) or phosphate buffered saline (PBS) (137 mM sodium chloride; 2,7 mM potassium chloride; 10 mM sodium monophosphate; 2 mM potassium diphosphate; pH=7,5±0,2).
- Flexible flocked or fiber swabs for collecting nasopharyngeal specimens.
- Sterile swab with viscose tip in individual package.
- Disposable tightly closed polypropylene 1.5-ml and 2.0-ml tubes for sampling and pretreatment.
- Sterile RNase-free pipette tips with aerosol filters (up to 10, 100, 200, 1000 and 5000 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge up to 12,000 g (suitable for Eppendorf tubes).
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir to throw off and inactivate the material.
- Disposable powder-free gloves and a laboratory coat.

For RNA extraction, reverse transcription and isothermal amplification

- Sterile RNase-free pipette tips with aerosol filters (up to 10, 100, 200 µl).
- Tube racks.
- RNA extraction kit.
- Vortex mixer.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany); CFX 96 (Bio-Rad, USA), QuantStudio 5 (Thermo, USA)).
- Disposable polypropylene tubes:
  a) screwed or tightly closed 1.5-ml tubes for reaction mixture preparation.
  b) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
  c) 96- or 384-well plate if a plate-type instrument is used
  d) thin-walled 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Disposable powder-free gloves and a laboratory coat.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use the reagent kit if the internal packaging was damaged or its appearance was changed.
- Do not use the reagent kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- Safety Data Sheets (SDS) are available on request.
- The reagent kit is intended for single use for analysis of specified number of samples (see the section “Content”).
- The reagent kit is ready for use in accordance with the Instruction Manual. Use the reagent kit strictly for intended purpose.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

Not for use in the Russian Federation

6. SAMPLING AND HANDLING

AmpliSens® SARS-CoV-2-IT reagent kit is intended for analysis of the RNA extracted with the use of RNA extraction kits from the biological material (nasopharyngeal and oropharyngeal swabs).

NOTE: It is recommended to combine nasopharyngeal and oropharyngeal swabs in a single tube. For this purpose, first take the swabs from the mucous membrane of inferior nasal meatus and oropharynx using different swabs and then place the ends of both shafts into one tube containing 500 µl of **Transport Medium for Storage and Transportation of Respiratory Swabs** (REF 959-CE, REF 957-CE, REF 958-CE) and analyze them as a single sample.

Sampling

Nasopharyngeal swabs. If the nasal cavity is full of mucus it is recommended to blow the nose before the procedure. Nasopharyngeal swabs are collected with sterile dry flocked swab with plastic shaft. Gently insert the swab along the external nasal wall to a depth of 2–3 cm towards the inferior nasal concha. Then move the swab slightly lower, insert it in the inferior nasal meatus under the inferior nasal concha, rotate, and remove along the external nasal wall. The total depth of insertion of the swab should be approximately half of the distance from the nostril to the ear hole (3-4 cm for children and 5-6 cm for adults).

When the material is obtained, insert the working part of the swab into a sterile disposable tube with 1.0 ml of **Transport Medium for Storage and Transportation of Respiratory Swabs** (REF 959-CE, REF 957-CE, REF 958-CE), the flexible part of the swab is folded up 3 times, then, covering the top of the tube with a lid, the handle of the swab is lowered down, achieving complete breaking off the end of shaft. Close and mark the tube with the solution and the swab.

It is allowable to use dry sterile polystyrol swabs with a viscose tip for collecting material from adults.

Oropharyngeal swabs are collected using sterile dry rayon swabs by rotating the swab over the surface of tonsils, palatine arches, and posterior wall of pharynx.

When the material is obtained, insert the working part of the swab into a sterile disposable tube with 500 µl of **Transport Medium for Storage and Transportation of Respiratory Swabs** (REF 959-CE, REF 957-CE, REF 958-CE). Break off the end of shaft to allow tight closing of tube cap. Close and mark the tube with the solution and the swab.

Nasopharyngeal and oropharyngeal swabs can be stored before pretreatment:

- at the temperature from 2 to 8 °C – for 3 days;
- at the temperature from minus 24 to minus 16 °C – for 1 year.

Only one freeze-thawing cycle is acceptable.

Pretreatment

Nasopharyngeal and oropharyngeal swabs. Vortex the tube for 5 s to sediment drops from the interior wall of the tube lid. If the liquid level in the tube is below 0.5 ml, the volume should be increased by adding 0.5 ml of saline solution, vortex the tube for 5 s to sediment drops from the interior wall of the tube lid. If there is mucus in the sample, **Mucolysin** (REF 180-CE) reagent should be added up to the mark of 1 ml. Incubate at the room temperature (from 18 to 25 °C) for 5 min (until visual clarification). In case of sedimentation centrifuge the samples at 600 rpm for 5 min. 100 µl of sample is used for RNA extraction. The residual sample should be frozen if it is necessary to repeat the analysis.

The pretreated samples can be stored before the analysis:

- at the room temperature – for 6 hours;
- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 24 to minus 16 °C – for 1 year;
- at the temperature not more than minus 68 °C – for a long time.

Interfering substances and limitations of using test material samples

Potential interfering substances

Endogenous and exogenous substances that may be present in the biological material (nasopharyngeal and oropharyngeal swabs) used for the study were selected to assess potential interference.

Model samples of biological material without adding and with the addition of potential interfering substances were tested (see Table 2).

Model samples contained Positive Control sample with SARS-CoV-2 RNA concentration of $1x10^4$ GE/ml.

Table 2

Test material	Type of potential interferent	Potential interferent	Tested concentration in a sample	Interference presence	
				RIBO-prep	MAGNO-sorb
Nasopharyngeal and oropharyngeal swabs	Endogenous substances	Mucin	6 mg/ml	Not detected	
			9 mg/ml	Detected	
		Hemoglobin	0.21 g/ml	Not detected	
	Exogenous substances	Lugol solution with glycerin	2.5 %	Not detected	
		Xylomethazoline	0.005 %	Not detected	
		Sodium chloride	0.045 %	Not detected	

7. WORKING CONDITIONS

AmpliSens® SARS-CoV-2-IT reagent kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8. PROTOCOL

8.1. RNA extraction

It is recommended to use the following nucleic acid extraction kit:

- **RIBO-prep**, REF K2-9-Et-100-CE;
- **MAGNO-sorb**, REF K2-16-200-CE, REF K2-16-1000-CE.

In the extraction procedure it is necessary to carry out the control reactions as follows:

C– Add **100 µl of C–** to the tube labelled C– (Negative Control of Extraction)

PCE Add **90 µl of C–** and **10 µl** of **C+ IT-SARS-CoV-2** to the tube labeled PCE (Positive Control of Extraction)

NOTE: Extract the RNA according to the manufacturer's protocol.

NOTE: Washing Solution 5 is not used when carrying out the extraction with MAGNO-sorb

NOTE: RNA extraction is performed from 100 µl of prepared samples of biological material.

It is recommended to carry out the RT-IT just after obtaining the RNA samples. It is allowed to store the RNA samples at the temperature from 2 to 8 °C for 30 min, at the temperature from minus 24 to minus 16 °C for 1 week and at the temperature ≤ –68 °C for 1 year. Only one freeze-thawing cycle is required.

8.2. Preparing reverse transcription and isothermal amplification

8.2.1 Preparing tubes for RT-IT

The total reaction volume is **20 µl**, the volume of the **RNA** sample is **10 µl**.

The type of tubes depends on the instrument used for analysis. Use disposable filter tips for adding reagents, RNA and control samples into tubes.

1. Calculate the required quantity of each reagent for reaction mixture preparation. For one reaction:
   - **5 µl** of **IT-mix SARS-CoV-2,**
   - **5 µl** of **IT-mix-E.**

   Prepare the reaction mixture for the total number of test and control samples plus two extra reactions. See numbers of control samples in item 7.

NOTE: Prepare the reaction mixture just before use.

2. Thaw and mix the tube with **IT-mix SARS-CoV-2**, sediment the drops by vortex. Mix the tube with **IT-mix-E** by pipetting, sediment the drops by vortex.

NOTE: The IT kit reagents can be stored at 2 to 8 °C if they are used up within 1 week. Discard the reagents after this time period.

3. In a new tube prepare the reaction mixture. Mix the required quantities of **IT-mix SARS-CoV-2** and **IT-mix-E**. **Mix thoroughly**, sediment the drops by vortex.
4. Take the required number of the tubes or strips for RT-IT of test and control samples.
5. Transfer **10 µl** of the prepared reaction mixture to each tube. Discard the unused reaction mixture.
6. Using tips with aerosol filter, add **10 µl** of **RNA samples** obtained at the RNA extraction stage.

NOTE: Avoid transferring the sorbent together with the RNA samples extracted with reagent kits for extraction by magnetic separation.

7. Carry out the control reactions:

PCE – Add **10 µl** of **the sample extracted from C+** to the tube labeled PCE (Positive Control of Extraction).

C– – Add **10 µl** of **the sample extracted from C–** to the tube labeled C– (Negative Control of Extraction).

To rule out possible contamination, carry out an additional control reaction for Negative Control of RT-IT:

NCA – Add **10 µl** of **C–** to the tube labelled NCA (Negative Control of RT-IT).

NOTE: Carry out RT-IT just after the mix of reaction mixture and RNA-samples and controls.

8.2.2. Amplification

1. Create a temperature profile on your instrument as follows:

Table 3

Amplification program

Step	Temperature, °C	Time	Cycles
1	37	5 min	1
2	65	30 s Fluorescence acquiring	50

Fluorescent signal is detected in the channel for the FAM fluorophore.

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin*.
3. Insert tubes into the reaction module of the device.

NOTE: It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them into the instrument.
Insert empty tubes at the edges of reaction module in case of incomplete filling of plate-type instrument.

4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

The curves of fluorescent signal accumulation indicating the amplification product accumulation are analyzed in one channel:

Table 4

Channel for the fluorophore	FAM
Amplification product	SARS-CoV-2 cDNA

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the RNA sample in the corresponding column of the results grid.

Principle of results interpretation is following:

Table 5

Results Interpretation

Ct value in the channel for the FAM fluorophore	Result
absent or > boundary value	SARS-CoV-2 RNA **is not detected**
< boundary value	SARS-CoV-2 RNA **is detected**

Quality control of analysis

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of extraction and RT-IT are correct (see Table 6).

Table 6

Results for controls

Control	Stage for control	*Ct* value in the channel for the FAM fluorophore
PCE	RNA extraction	< boundary value
C–	RNA extraction	absent or > boundary value
NCA	RT-IT	absent or > boundary value

NOTE: Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the kit.

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. The *Ct* value determined for the Positive Control of Extraction (PCE) is greater than the boundary value or absent. The analysis (beginning with the RNA extraction stage) should be repeated for all samples.
2. If the *Ct* value determined for the Negative Control of Extraction (C–) is less than the boundary value. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of analysis. Measures for detecting and elimination of contamination source must be taken. The analysis (beginning with the RNA extraction stage) should be repeated for all samples in which specific RNA was detected.
3. If the *Ct* value determined for the Negative Control of RT-IT (NCA) is less than the boundary value. The contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of analysis. Measures for detecting and elimination of contamination source must be taken. The amplification and detection should be repeated for all samples in which specific RNA was detected.

NOTE: The IT process produces more amplification products than PCR. Extreme caution is required when working with IT products

4. The *Ct* value is determined for the test sample, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to check the correctness of selected threshold line level or parameters of base line calculation. If the result has been obtained with the correct level of threshold line (base line), the amplification and detection should be repeated for this sample.

If you have any further questions or if you encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

AmpliSens® SARS-CoV-2-IT reagent kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® SARS-CoV-2-IT** reagent kit are to be stored at the temperature from minus 24 to minus 16 °C when not in use. All components of the **AmpliSens® SARS-CoV-2-IT** reagent kit are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: IT-mix-E is to be kept away from light

13. SPECIFICATIONS

13.1. Limit of detection

Test material	Nucleic acid extraction kit	Amplification kit	Limit of detection, GE (copies)/ml
Nasopharyngeal and oropharyngeal swabs	RIBO-prep, MAGNO-sorb	IT kit variant 200	$1x10^3$
			$5x10^3$

Note – one copy of cDNA target corresponds to one genomic equivalent (genome, GE) of the virus.

The claimed features are achieved while respecting the rules specified in the section "*Sampling and Handling".*

13.2. Analytical specificity

The analytical specificity of **AmpliSens® SARS-CoV-2-IT** reagent kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The reagent kit detects a fragment of SARS-CoV-2 RNA (clinical samples with SARS-CoV-2 RNA in concentration no less than $1x10^3$ GE (copies)/ml, the specificity was confirmed by direct sequencing of nucleotide sequences).

The analytical specificity was proved when investigating the RNA/DNA of the following microorganisms/strains in concentration no more than $1x10^9$ GE/ml and no less than $1x10^5$ GE/ml:

- strains from ATCC (American Type Culture Collection, USA): *Streptococcus pneumoniae* ATCC® 49619™, *Staphylococcus epidermidis* ATCC® 12228™, *Staphylococcus haemolyticus* ATCC® 29970™, *Streptococcus agalactiae* ATCC® 13813™, *Enterococcus faecalis* ATCC® 19433™, *Proteus mirabilis* ATCC® 12453™, *Pseudomonas aeruginosa* ATCC® 9027™, *Legionella pneumophila* ATCC® 33152™, *Staphylococcus aureus* (*MRSA*) ATCC® 43300™, *Escherichia coli* ATCC® 25922™, *Klebsiella pneumoniae* ATCC® 27736™, *Enterobacter cloacae* ATCC® 13047™, *Bordetella pertussis* ATCC® 9340™, *Moraxella catarrhalis* ATCC® 25240™, *Haemophilus influenzae* ATCC® 9006™, *Pneumocystis jirovecii* ATCC® PRA-159™, *Candida albicans* ATCC® 14053™, *Human adenovirus* 1 ATCC® VR-1™, *Human rhinovirus* ATCC® VR-1663™, *Human respiratory syncytial virus* ATCC® VR-1540™, *Human herpesvirus* ATCC® VR-260™, *Human enterovirus* 71 ATCC® VR-1432™;
- clinical samples (the specificity was confirmed by direct sequencing of nucleotide sequences): *Yersinia enterocolitica*, *Saccharomyces cerevisiae*, SARS-CoV, MERS-CoV, *Influenza virus* A, *Influenza virus* B, *Cytomegalovirus* (*CMV*), *Epstein-Barr virus* (*EBV*), *Human parainfluenza virus* 1-4, *Human bocavirus*, *Human metapneumovirus* (*hMPV*);
- human DNA in concentration of 10 µl/ml.

The nonspecific reactions were absent while testing RNA/DNA samples of the above-mentioned microorganisms and human DNA.

The clinical specificity of **AmpliSens® SARS-CoV-2-IT** reagent kit was confirmed in laboratory clinical trials.

The information about interfering substances is specified in the *Interfering substances and limitations of using test material samples*.

13.3. Repeatability and reproducibility

Repeatability and reproducibility were determined by testing of positive and negative model samples.

Repeatability conditions included testing in the same laboratory, by the same operator, using the same equipment within a short period of time. Reproducibility conditions included testing different lots of reagent kit in different laboratories, by different operators, on different days, using different equipment. The results are presented in Table 7.

Table 7

Sample type	Repeatability		Reproducibility	
	Number of repeats	Agreement of results, %	Number of repeats	Agreement of results, %
Positive	10	100	40	100
Negative	10	100	40	100

Trueness, measurement range, biological reference interval are not applicable for this reagent kit.

13.4. Diagnostic characteristics

Table 8

Diagnostic characteristics of AmpliSens® SARS-CoV-2-IT reagent kit

Test material	Diagnostic sensitivity[1] (with a confidence level of 95 %)	Diagnostic specificity[2] (with a confidence level of 95 %)
Nasopharyngeal and oropharyngeal swabs	100 (96.4 – 100) %	100 (96.4 – 100) %

14. REFERENCES

1. Park GS, Ku K, Baek SH, et al. Development of Reverse Transcription Loop-Mediated Isothermal Amplification Assays Targeting Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *J Mol Diagn*. 2020;22(6):729-735. doi:10.1016/j.jmoldx.2020.03.006.
2. Jiang M, Pan W, Arasthfer A, et al. Development and Validation of a Rapid, Single-Step Reverse Transcriptase Loop-Mediated Isothermal Amplification (RT-LAMP) System Potentially to Be Used for Reliable and High-Throughput Screening of COVID-19. *Front Cell Infect Microbiol*. 2020;10:331. Published 2020 Jun 16. doi:10.3389/fcimb.2020.00331.
3. Yüce M, Filiztekin E, Özkaya KG. COVID-19 diagnosis - A review of current methods. *Biosens Bioelectron*. 2021;172:112752. doi:10.1016/j.bios.2020.112752.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® SARS-CoV-2-IT** reagent kit has been tested against predetermined specifications to ensure consistent product quality.

AmpliSens®

EC REP

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[1] Relative sensitivity in comparison with applied reference assay.
[2] Relative specificity in comparison with applied reference assay.