REVERTA-L RT reagents kit

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code	Σ	Contains sufficient for <n> tests
IVD	*In vitro* diagnostic medical device		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit	EC REP	Authorized representative in the European Community
	Manufacturer		Date of manufacture

1. INTENDED USE

REVERTA-L RT reagents kit is intended for cDNA synthesis from an RNA template for subsequent analysis by the polymerase chain reaction (PCR).

Indications and contra-indications for use of the reagent kit
The reagent kit is used for RNA reverse transcription as a preanalytical stage in clinical laboratory diagnostics.

2. PRINCIPLE

The synthesis of cDNA from an RNA template extracted from the test biomaterial is carried out using the enzyme reverse transcriptase (revertase) and short oligonucleotides (hexamers) with a random sequence as primers for polymerization (random primers). As a result of the reverse transcription reaction, cDNA is synthesized from all RNA present in the test sample.

3. CONTENT

REVERTA-L RT reagents kit is produced in 2 forms:
variant 50 REF K3-4-50-CE,
variant 100, REF K3-4-100-CE.
Variant 50 or 100 include:

Reagent	Description	variant 50		variant 100	
		Volume, ml	Quantity	Volume, ml	Quantity
RT-G-mix-1	colorless clear liquid	0.01	5 tubes	0.01	10 tubes
RT-mix	colorless clear liquid	0.125	5 tubes	0.125	10 tubes
Revertase (MMlv)	colorless clear liquid	0.03	1 tube	0.06	1 tube
DNA-buffer	colorless clear liquid	1.2	1 tube	1.2	2 tubes

Variant 50 is intended for 60 reverse transcription reactions, including controls.

Variant 100 is intended for 120 reverse transcription reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- PCR box or Biological cabinet.
- Thermostat with working temperature 25 °C to 100 °C (suitable for Eppendorf tubes).
- Vortex mixer.
- Pipettes (adjustable).
- 0.2 (0.5) ml polypropylene sterile tubes.
- Disposable RNase-free and DNase-free pipette tips with aerosol filters (up to 200 µL).
- Tube racks.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Disposable powder-free gloves and a laboratory coat.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile RNase-free pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (samples, controls and amplicons) away from all other reagents.
- 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 a kit after its expirationdate.
- Dispose of all samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucose membranes. If skin, eyes and mucose membranes contact immediately flush with water, seek medical attention.
- Safety Data Sheets (SDS) are available on request.
- 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.

6. SAMPLING AND HANDLING

The material for the reverse transcription reaction is an RNA solution obtained earlier at the extraction stage from the test material
The information about the procedure for sampling, transportation and storage, the necessity of pretreatment and procedure of RNA extraction are specified in the Instruction Manual of the PCR kit.

Interfering substances and limitations of using test material samples
The information about potential interfering substances and limitations of using test material samples is specified in the Instruction Manual of the PCR kit.

7. WORKING CONDITIONS

REVERTA-L RT reagents 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's recommended to use AmpliSens RNA extraction kits.

8.2. Reverse Transcription Reaction

Total reaction volume – **20 µl**, volume of RNA sample - **10 µl**.

1. Prepare required number of 0.2 (0.5) ml disposable polypropylene microcentrifuge tubes.
2. Prepare reaction mixture for 12 reactions.
   2.1. Add **5 µl** of **RT-G-mix-1** to the tube containing **RT-mix**, carefully vortex the tube, and then centrifuge, make sure that there are no drops on the caps of the tubes.
   2.2. Add **6 µl** of **Revertase (MMlv)** to the tube with reagent mix, then pipette 5 times, vortex the tube, and then centrifuge, make sure that there are no drops on the caps of the tubes.
3. Add **10 µl** of prepared reaction mixture into each test tube.
4. Add **10 µl** of **RNA-sample** to the tubes with reaction mixture. Carefully mix by pipetting.
5. Place the test tubes into thermostat and incubate at 37 °C for 30 minutes.
6. Dilute the cDNA sample obtained at the reverse transcription reaction in the ration 1:1 with DNA-buffer. To do that add **20 µl DNA-buffer** to **20 µl of cDNA sample**. Carefully mix by pipetting 10 times.

cDNA samples can be stored at the temperature from minus 24 to minus 16 °C for a week or at the temperature not more than minus 68 °C for a year.

8.3. Amplification

It's recommended to use of AmpliSens RT-PCR amplification kits.

NOTE: Please carry out the reverse transcription reaction and amplification according to the Instruction Manual of PCR kit for RNA detection of analyzed pathogen. The volume of the used reagents as well as the order of reaction steps can vary.

9. TROUBLESHOOTING

These troubleshooting guides may be helpful in explaining any problem that may arise.

False negatives with extracted product:

- Loss of the enzyme activity. It is necessary to store the test-tube containing enzymes at the labeled temperature.
- Errors during the dispensing of the extracted RNA. It is necessary to monitor the dispensing of extracted RNA carefully.
- Degradation of the nucleic acid contained in the sample. It is necessary to use a new sample, store samples appropriately.
- Errors in the thermal cycle settings. It is necessary to check the thermal cycle setting on the thermal cycler.
- Degradation of the extracted nucleic acid. Use plastic free from DNAses and RNAses. Change the aliquot used for components of kit.

False positives with extracted product:

- Errors at the beginning of the reaction. Open one test tube at a time, avoid spilling the contents of the test tube, and always change tips.
- Contamination of the reagents prepared for the step. Prepare a new aliquot of components.
- Contamination with amplicons in the designated of nucleic acid extraction area. Clean surfaces and instruments using aqueous detergents, wash lab coats, replace test tubes and tips in use.

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

10. TRANSPORTATION

REVERTA-L RT reagents kit should be transported at 2–8 °C for no longer than 5 days.

11. STABILITY AND STORAGE

All components of the **REVERTA-L** RT reagents kit are to be stored at the temperature from minus 24 to minus 16 °C when not in use. All components of the **REVERTA-L** RT reagents 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.

12. REFERENCES

1. Chomczynski P. and Sacchi N. Anal.Biochem 1987, V.162, P.156-159.

13. QUALITY CONTROL

In accordance with Federal Budget Institution of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Total Quality Management System, each lot of **REVERTA-L** RT reagents kit has been tested against predetermined specifications to ensure consistent product quality.

Please contact our Authorized representative in the European Community if side effects, undesirable reactions, facts and circumstances that pose a threat to the life and health of citizens and medical workers are identified during the use of the reagent kit.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
27.12.10 KM	Cover page	The phrase “For Professional Use Only” was added
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Stability and Storage	The information about the shelf life of open reagents was added
	Key to Symbols Used	The explanation of symbols was corrected
27.06.11 VV	Cover page, text	The name of Institution was changed to Federal Budget Institution of Science “Central Research Institute for Epidemiology”
01.11.16 PM	7. Working conditions	The name of the reagents kit was corrected
09.04.20 MM	Through the text	The text formatting was changed
	Footer	The phrase “Not for use in the Russian Federation” was added
12.03.21 VA	—	The name, address and contact information for Authorized representative in the European Community was changed
17.09.21 MM	1. Intended use	The section was actualized
	2. Principle	The section was actualized
	4. Additional requirements	The section was actualized
	6. Sampling and handling. Interfering substances and limitations of using test material samples	The information about interfering substances and limitations of using test material samples was added
	12. References	The reference to the Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics” was deleted
31.05.22 EM	2. Principle	The information about the preanalytical stage was moved to “Indications and contra-indications for use of the reagent kit” subsection
	6. Sampling and handling	The name of section was changed, the information about the interfering substances and limitations of using test material samples was moved to a separate subsection
	13. Quality control	The Authorized representative in the European Community was specified for the contact in case of undesirable effects when using the reagent kit

AmpliSens®

EC REP

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