

REVERTA-L RT reagents kit



For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

	Catalogue number		Caution
	Batch code		Contains sufficient for <n> tests
	<i>In vitro</i> diagnostic medical device		Use-by Date
	Version		Consult instructions for use
	Temperature limit		Authorized representative in the European Community
	Manufacturer		Date of manufacture

1. INTENDED USE

REVERTA-L RT reagents kit is intended for complementary DNA (cDNA) synthesis from RNA extracted from biological samples.

2. PRINCIPLE

The procedure comprises the execution of reverse transcription reaction using random sequence hexamers (random primers) as polymerization primers. The reverse transcription reaction generates cDNAs from all the different RNA molecules present in RNA extracted from the sample being tested. cDNAs can be used in diagnostic tests based on amplification reactions. With an amplification assay it is possible to verify the presence in the reverse transcription reaction product of specific cDNA target molecules, originating for example from genomic regions of microorganisms with an RNA genome.

3. CONTENT

REVERTA-L RT reagents kit is produced in 2 forms:

variant 50, K3-4-50-CE,

variant 100, K3-4-100-CE.

Variant 50 or 100 includes:

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 (MMIv)	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

- Disposable powder-free gloves.
- Pipettes (adjustable).
- Disposable RNase-free and DNase-free pipette tips with aerosol filters (up to 200 µL).
- Vortex mixer.
- Desktop microcentrifuge with rotor for 2 ml reaction tubes (RCF max. 16,000 x g).
- PCR box or Biological cabinet.
- Vacuum aspirator with flask for removing supernatant.
- Tube racks.
- Thermocycler.
- 0.2 (0.5) ml polypropylene sterile tubes.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Waste bin for used tips.
- Permanent pen for labeling.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile RNase-free pipette tips with aerosol filters and use new tip for every procedure.
- Store 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 gloves, laboratory coats, 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 expiry date.
- 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 the techniques of DNA amplification.
- The laboratory process must be one directional; it should begin in the Extraction Area move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.

6. SAMPLING AND HANDLING

Obtaining samples of biological materials for PCR-analysis, transportation and storage are described in manufacturer's handbook [2]. It is recommended that this handbook is read before starting work. RNA solution is a sample for study in reverse transcription reaction.

7. WORKING CONDITIONS

REVERTA-L RT reagents kit should be used at 18–25 °C.

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 ready-to-use reagent mix for 12 reactions.
 - 2.1. Add 5 µl of RT-G-mix-1 to the tube containing RT-mix, carefully mix on vortex for 3 s, centrifuge for 5-7 s (for removing drops from the internal surface of the test tubes caps).
 - 2.2. Add 6 µl of Revertase (MMIv) into the tube with reagent mix, then pipette 5 times and mix on vortex for 3 s, and then centrifuge for 5-7 s (to remove any drop adhering to the internal surface of the test tubes caps).
 3. Dispense 10 µl of ready-to-use reagent mix into each prepared test tube.
 4. Add 10 µl RNA-sample to the appropriate test tube with ready-to-use reagent mix. Carefully mix, using the pipette.
 5. Place the test tubes into thermocycler and incubate at 37 °C for 30 minutes.
 6. Dilute each cDNA sample in the ration 1:1 with DNA-buffer. To do that, add 20 µl DNA-buffer to each test tube. Carefully mix, using the pipette (10 times).
- cDNA samples can be stored at the temperature not more than 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 manufacturer's instructions of specific reagents kit. The volume of the reagents used as well as the order of 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 must be 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.
2. Handbook "Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics", developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being.

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.

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

AmpliSens®



Ecoli s.r.o., Studenohorská 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040



Federal Budget Institute of
Science "Central Research
Institute for Epidemiology"
3A Novogireevskaya Street
Moscow 111123 Russia