CE

IVD

For Professional Use Only

REVERTA-L

RT reagents kit

Instruction Manual

AmpliSens®

EC REP

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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:

REVERTA-L RT reagents kit variant 50, **REF** K3-4-50-CE

REVERTA-L RT reagents kit variant 100, **REF** K3-4-100-CE

REVERTA-L RT reagents kit 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 (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

REVERTA-L RT reagents kit variant 50 is intended for 60 reverse transcription reactions, including controls.

REVERTA-L RT reagents kit 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 for ≤ –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.
- Material Safety Data Sheets (MSDS) 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

DNA-sorb-AM nucleic acid extraction 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 (MMlv)** 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 ≤ –16 °C for a week or at ≤ –68 °C for a year.

8.3. Amplification.

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

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 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 temperature from minus 24 to minus 16 °C when not in use. All components of the 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. Manual “Sampling, transportation and storage of clinical material for PCR diagnostics”, developed by Federal State Institution of Science “Central Research Institute of Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.

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 is tested against predetermined specifications to ensure consistent product quality.

14. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code	Σ	Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation	EC REP	Authorised representative in the European Community
	Manufacturer		Date of manufacture

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”