

# RNA RETROTRANSCRIPTION KIT



Kit for the Reverse Transcription of viral RNA and m-RNA with Random Hexamers

4BShopLab

## RNA Retrotranscription Kit

### A. INTENDED USE

The **RNA Retrotranscription kit** coded **RNART.CE** is intended for the reverse transcription to cDNA of highly purified RNA coming from Virus (vRNA) with Random Hexamers.

### B. INTRODUCTION

Reverse transcription polymerase chain reaction (RT-PCR) is aimed to the generation of many copies of a cDNA from an RNA molecule using the enzyme Reverse Transcriptase.

The resulting cDNA can be used as template of a traditional or real-time PCR.

### C. PRINCIPLE OF THE TEST

**Viral RNA**, recovered from the biological sample under investigation through an extraction step, is retrotranscribed into cDNA using the reagents supplied in the kit.

### D. REAGENTS

The standard format of the product code **RNART.CE** contains reagents for 25 tests.

Reagent	Labelling and Contents	RNART.CE 25 Reactions
<b>REAGENT A</b> CODED: RNART/ A  COLOR CODE: YELLOW	<b>MMLV ENZYME</b>	1vial/35 µl
<b>REAGENT B</b> CODED: RNART/ B COLOR CODE: ORANGE	<b>RETROMIX</b>	1vial/0.285ml
<b>REAGENT C</b> CODED: ALL/ C COLOR CODE: RED	<b>MB GRADE WATER</b>	1vial/1.5 mL
<b>REAGENT D</b> CODED: RNART/ D COLOR CODE: VIOLET	<b>RANDOM HEXAMERS</b>	1vial/30 µl
Package Insert	<b>Instruction for Use</b>	1

**Important note:** Upon request, Dia.Pro can supply reagents for 50 tests, as reported below :

1. Reagent A	n°2 vials/35 µl
2. Reagent B	n°2 vials/0.285 ml
3. Reagent C	n°1 vials/1.5 ml
4. Reagent D	n°2 vials/30 µl
5.Pack. insert	n°1
<b>Number of tests</b>	<b>50</b>
<b>Code</b>	<b>RNART.CE.50</b>

### E. MATERIALS REQUIRED BUT NOT PROVIDED

1. Calibrated Micropipettes
2. RNA Purification kit
3. Microcentrifuge
4. Tube racks
5. Sterile filtered tips with aerosol barrier
6. 0,2 ml Microtubes recommended from the PCR instruments manufacturers
7. Disposable gloves, powder-free
8. Programmable Thermalcycler
9. Absorbent paper tissues.
10. Vortex or similar mixing tools.

### F. WARNINGS AND PRECAUTIONS

1. The kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
2. The technical personnel must be deeply trained in the use of thermalcyclers, in the manipulation of Molecular Biology reagents and skilled in the PCR amplification protocols.
3. All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses. The use of any sharp (needles) or cutting (blades) devices should be avoided. All the personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
4. All the personnel involved in sample handling should be vaccinated for HBV and HAV, for which vaccines are available, safe and effective.
5. The laboratory environment should be controlled so as to avoid contaminants such as dust or air-born microbial agents, when opening kit vials
6. Upon receipt, store the kit at -15...-35°C into a temperature controlled refrigerator or cold room.
7. Do not interchange reagents between different lots of the kits. It is recommended that reagents between two kits of the same lot should not be interchanged.
8. Check that the reagents are clear and do not contain visible heavy particles or aggregates. If not, advise the laboratory supervisor to initiate the necessary procedures for kit replacement.
9. Avoid cross-contamination between samples by using disposable tips and changing them after each sample.
10. Avoid cross-contamination between kit reagents by using disposable tips and changing them between the use of each one.
11. Do not use the kit after the expiration date stated on the external container and internal (vials) labels.
12. Treat all specimens as potentially infective. All human serum specimens should be handled at Biosafety Level 2, as recommended by the Center for Disease Control, Atlanta, U.S. in compliance with what reported in the Institutes of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories", ed. 1984.
13. Carry on all the working operations as quickly as possible maintaining the reagents on ice or in a cooling block.
14. Workflow in the laboratory must proceed in an unidirectional way, beginning in the Extraction Area and moving to the Amplification and Data Analysis Area. Do not return samples, equipment and reagents to the area where previous step have been performed. Never introduce an amplification product in the area designed for extraction/preparation of amplification products.
15. The use of disposable plastic-ware is recommended in the preparation of the liquid reagents or in transferring reagents into automated workstations, in order to avoid cross contamination.
16. Waste produced during the use of the kit has to be discarded in compliance with national directives and laws concerning laboratory waste of chemical and biological substances. In particular, liquid waste generated from the

washing procedure, from residuals of controls and from samples has to be treated as potentially infective material and inactivated before waste. Suggested procedures of inactivation are treatment with a 10% final concentration of household bleach for 16-18 hrs or heat inactivation by autoclave at 121°C for 20 min..

17. Accidental spills from samples and operations have to be adsorbed with paper tissues soaked with household bleach and then with water. Tissues should then be discarded in proper containers designated for laboratory/hospital waste.

18. Other waste materials generated from the use of the kit (example: tips used for samples and controls, used microplates) should be handled as potentially infective and disposed according to national directives and laws concerning laboratory wastes.

#### G. SPECIMEN: PREPARATION AND RECOMMENDATIONS

- Blood has to be drawn aseptically by venepuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis.
- No influence has been observed in the preparation of the sample with citrate, EDTA.  
Attention: Heparin ( $\geq 10$  IU/ml) affects the PCR reactions.  
Samples, which has been collected in tubes containing heparin as an anticoagulant should not be used. Also, samples of heparinised patients must not be used.
- Avoid any addition of preservatives to samples.
- Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. When the kit is used for the screening of blood units, bar code labeling and electronic reading is strongly recommended.
- Haemolysed (red) and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
- Sera, if not used immediately, must be divided in aliquots and stored at  $-20^{\circ}\text{C}$ .. $-80^{\circ}\text{C}$  after collection. Samples can be stored frozen at  $20^{\circ}\text{C}$ .. $-80^{\circ}\text{C}$  for several months. Any frozen samples should not be frozen/thawed more than once as this may affect the test result.
- Do not use heat inactivated samples as they could give origin to false reactivity.

#### H. PREPARATION OF REAGENTS AND WARNINGS

All of the reagents are **ready to use**.

Before using all components of the kit should be briefly centrifuged.

#### I. INSTRUMENTS AND TOOLS USED IN COMBINATION WITH THE KIT

- Micropipettes** have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of  $\pm 5\%$ . Decontamination of spills or residues of kit reagents should also be carried out regularly.
- Extraction Device:** The RNART.CE Kit is intended to be used in combination only with quality Extraction Kits. The end users must strictly follow the Instruction for use supplied by the manufacturer.
- Thermo-cyclers.** The RNART.CE Kit is intended to be used in combination with the Real Time Thermal cyclers or normal thermal cyclers.  
The end users must strictly follow the Instruments Instruction for use supplied by the manufacturers.

#### L. PRE ASSAY CONTROLS AND OPERATIONS

- Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
- Check that the liquid reagents are not contaminated by naked-eye visible particles or aggregates. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box.
- Turn the Thermo-cyclers on, check settings and be sure to use the right assay protocol.
- Follow strictly the Instruments Manual supplied by the manufacturers for the correct setting of the Thermo-cyclers.
- Check that the micropipettes are set to the required volume.
- Check that all the other equipment is available and ready to use.
- In case of problems, do not proceed further with the test and advise the supervisor.

#### M. ASSAY PROCEDURE

The assay has to be carried out according to what reported below.

##### M.1 RNA extraction

The RNART.CE Kit is intended for the use in combination only with quality Extraction Kits. The end users must strictly follow the Instruction for use supplied by the manufacturer.

##### M.3.1 Preparing the Retro-transcription

- Thawing all of the reagents described in Section D;
- Prepare the required number of reaction tubes or plate for the samples under evaluation and for the Control when it is possible
- Consider that the samples should be tested in duplicate;
- Include at least 1 tube for the ALL/C (negative control)
- Prepare the **Reverse Transcription Mix**

#### Preparation of the Reverse Transcription Mix (RETRO Mix) with RANDOM HEXAMERS

Number of Reactions		1	25
<b>A</b>	MMULV	1 $\mu\text{l}$	25 $\mu\text{l}$
<b>B</b>	Retro mix	9.5 $\mu\text{l}$	237.5 $\mu\text{l}$
<b>C</b>	MB WATER	8,5 $\mu\text{l}$	212.5 $\mu\text{l}$
<b>D</b>	Random Hexamers	1 $\mu\text{l}$	25 $\mu\text{l}$
<b>Tot vol.</b>		<b>20 <math>\mu\text{l}</math></b>	<b>500 <math>\mu\text{l}</math></b>

##### M.3.2 Reverse Transcription procedure

##### Preparation of PCR assay

Number of Reactions	1
<b>RETRO Mix</b>	20 $\mu\text{l}$
<b>Sample</b>	10 $\mu\text{l}$
<b>Tot vol.</b>	<b>30 <math>\mu\text{l}</math></b>

- Add the **Samples** to the reaction tubes.

- Close firmly the reaction tubes
- Centrifuge briefly the reaction tubes at 2000rpm
- Load the tubes in the Thermo-cycler Thermoblock Holder.
- After the setting operations described in the Sections M5 start the Thermo-cycler run.

### M.5 Instrument programming

For programming the instrument refer to the Instruction Manual provided by the manufacturers.

#### M.5.1 Thermal Profile

The thermal profile is reported in the table below:

Step	Cycle	Temp.	Time
1	1	25°C	10 min
2	1	42°C	60 min
3	1	85°C	5 min

Set up the Thermacycler with the correct Thermal Profile following the Instruments Manual supplied by the manufacturer.

If you do not use immediately the cDNA you can keep the samples for 24 hr at 2°C...+8°C, for long time storage (1 or 2 weeks) the c-DNA has to be kept at -15°C...-35°C.

### O. INTERNAL QUALITY CONTROL AND TROUBLESHOOTING

A control of the quality of the reaction can be done only after the amplification step. The following results are possible:

#### Troubleshooting table

	Result	Possible Causes	CHECK
SAMPLE unknown	FALSE POSITIVE	<i>Error during RNA pipetting</i>	<ul style="list-style-type: none"> <li>• That one test-tube at time is opened avoiding spilling the contents</li> <li>• That any time the tips has been changed</li> </ul>
		<i>Contamination of the Reagents prepared in the session</i>	<ul style="list-style-type: none"> <li>• That the reagents dispensation has been monitored</li> <li>• That any time the tips has been changed</li> </ul>
		<i>Contamination of the AREA designed for the extraction/preparation of the amplification reactions.</i>	<ul style="list-style-type: none"> <li>• That the surfaces and tools are cleaned according how described in section F.</li> </ul>
		<i>Excess of Extracted RNA in the reaction</i>	<ul style="list-style-type: none"> <li>• That the concentration of extracted RNA added to Reaction tube has been correctly quantified</li> <li>• Do not exceed the concentration of 40 ng/ul (a total of 1 ug RNA) in the Reverse Transcription</li> </ul>
SAMPLE unknown	FALSE NEGATIVE	<i>Excess of cDNA or Reverse Transcription reagents in the amplification</i>	<ul style="list-style-type: none"> <li>• That has been avoided adding an exceeding quantity of Reverse Transcription reaction product into the amplification reaction</li> </ul>
		<i>Error during dispensation</i>	<ul style="list-style-type: none"> <li>• That the dispensation has been carefully done</li> </ul>
		<i>Loss of activity of the enzymes</i>	<ul style="list-style-type: none"> <li>• That the tubes has been kept on Ice</li> </ul>
		<i>Error in the Thermal Profile setting</i>	<ul style="list-style-type: none"> <li>• That the Thermal profile set on the thermal cycler has been checked before</li> </ul>

			the experiment starting
		<i>Degradation of the extracted Nucleic Acid</i>	<ul style="list-style-type: none"> <li>• That DNase and RNase-Free plastic has been used</li> <li>• That all the Good Laboratory Procedures for RNA handling has been strictly followed</li> </ul>
		<i>Bad quality of RNA extraction</i>	<ul style="list-style-type: none"> <li>• Repeat the Extraction step with a new sample</li> </ul>

If the results of the test match the CORRECT RESULT requirements stated above, proceed to the amplification step following the specific protocol design.

If one of more of the problems described in the table above happen, after checking, report any residual problem to the supervisor for further actions.

### Q. PERFORMANCES











Evaluation of Performances has been conducted in accordance to what reported in the Internal Technical Specifications or ITS.

## R. BIBLIOGRAPHY

**Perez-Novo, C.A. et al.** Impact of RNA quality on reference gene expression stability. *Biotechniques* 39, 52,54,,56 (2005)

**Gargano N; Cattaneo A; MRC Laboratory of Molecular Biology, Cambridge, UK.** Inhibition of murine leukaemia virus retrotranscription by the intracellular expression of a phage-derived anti-reverse transcriptase antibody fragment. *J Gen Virol.* 1997 Oct;78 ( Pt 10):2591-9

## 5. Symbols

LEGENDA			
	Product code		Storage temperature
	In Vitro Diagnostic Device		See use instructions
	Lot number		Manufacturer
	Expiry date		Number of tests
	CE conformity mark		Date of manufacturing




All the IVD Products manufactured by the company are under the control of a certified Quality Management System in compliance with ISO 13485 rule. Each lot is submitted to a quality control and released into the market only if conforming with the EC technical specifications and acceptance criteria.





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