HDV RNA RETROTRANSCRIPTION KIT

Kit for the Reverse Transcription of Hepatitis D Virus (HDV) RNA

4BShopLab

Rev. 2

HDV RNA Retrotranscription Kit

A. INTENDED USE

The **HDV RNA Retrotrascription kit** coded **RNART.HDV.CE** is intended for the reverse transcription to cDNA of Hepatits delta virus RNA.

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 use as template of a real-time PCR using the DRNA.CE kit (Dia.Pro)

C. PRINCIPLE OF THE TEST

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

D. REAGENTS

The standard format of the product code RNART.HDV.CE contains reagents for 50 tests.

Reagent	Labelling and Contents	RNART.CE 50 Reactions
REAGENT A **CODED: RNART/ A** **COLOR CODE: YELLOW**	**MMLV ENZYME**	2vial/35 µl
REAGENT B **CODED: RNART/ B** **COLOR CODE: ORANGE**	**RETROMIX**	2vial/0.285ml
REAGENT C **CODED: ALL/C** **COLOR CODE: RED**	**MB GRADE WATER**	2vial/1.5 mL
Package Insert	**Instruction for Use**	1

Important note: Upon request, Dia.Pro can supply reagents for 25,100 tests, as reported below **:**

1. Reagent A 2. Reagent B 3. Reagent C 4. Pack. insert	n°1 vials/35 µl n°1 vials /0,285 ml n°1 vials /1,5 ml n°1	n°4 vials/35 µl n°4 vials /0,285 ml n°3 vials /1,5 ml n°1
Number of tests	**25**	**100**
Code	**RNART.HDV.CE.25**	**RNART.HDV.CE.100**

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. 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 label.
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

1.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.
2. No influence has been observed in the preparation of the sample with citrate, EDTA.
Attention: Heparin (≥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.
3. Avoid any addition of preservatives to samples.
4. 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.
5. 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.
6. Sera, if not used immediately, must be divided in aliquots and stored at -20°..-80°C after collection. Samples can be stored frozen at 20°..-80°C for several months. Any frozen samples should not be frozen/thawed more than once as this may affect the test result.
7. 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

1. **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 +/-5%. Decontamination of spills or residues of kit reagents should also be carried out regularly.
2. **Extraction Device:** The RNART.HDV.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.
3. **Thermo-cyclers.** The RNART.HDV.CE Kit is intended to be used in combination preferably with Real Time PCR Thermal cyclers or PCR thermal cyclers only if correctly calibrated and maintained.
   The end users must strictly follow the Instruments Instruction for use supplied by the manufacturers.

L. PRE ASSAY CONTROLS AND OPERATIONS

1. Check the expiration date of the kit printed on the external label of the kit box. Do not use if expired.
2. 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.
3. Turn the Thermo-cyclers on, check settings and be sure to use the right assay protocol.
4. Follow strictly the Instruments Manual supplied by the manufacturers for the correct setting of the Thermo-cyclers.
5. Check that the micropipettes are set to the required volume.
6. Check that all the other equipment is available and ready to use.
7. 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 Viral RNA extraction

The extraction step of the HDV genomic RNA has to be carried out exclusively in combination with the following kit:

Material	Description	Kit code	manufacturer
Serum/Plasma	QIAamp Viral RNA mini kit®	52906	Qiagen™

The RNA isolation must be carried out only according to the Manufacturer's instructions.

M.2 Preparing the Retro-trasciption

IMPORTANT: Carry on all the following working operations as quickly as possible maintaining the reagents on ice or in a cooling block

- Thaw the Retrotrascription mix (REAGENT B) just before the use.
- Thaw the REAGENT C (ALL/C) just before the use.
- All the Reagents have to be briefly centrifuged before the use and kept in the Cooling Block.
- Prepare the **RETROMIX** as follow:

Preparation of the RETROMIX

Number of Reactions		1	25
REAGENT A (RNART.CE)	MMULV	1 µl	25 µl
REAGENT B (RNART.CE)	Retro mix	9.5 µl	237.5 µl
REAGENT C (RNART.CE)	MB WATER	8.5 µl	212.5 µl
HDV/REV (*)	Specific Reverse Primer	1 µl	25 µl
Tot vol.		**20 µl**	**500 µl**

(*) Important Note: The Reagent Specific Reverse Primer (HDV/REV) is included in the kit DRNA.CE

Preparation of Retrotranscription PCR assay

The reverse transcription of HDV RNA should be performed as follows:

Number of Reactions	1
RETROMIX	20 µl
Sample (RNA) or ALL/C	10 µl
Tot. vol.	**30 µl**

- 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)
- 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 following Sections start the Thermo-cycler run.

Step	Cycle	Temp.	Time
1	1	42°C	60 min
2	1	99°C	10 min

Set up the Thermacycler with the correct Thermal Profile following the Instruments Manual supplied by the manufacturer.
At the end of the Retrotranscrition phase the samples must be stored at -20°C.

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.

N. 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	*Error during RNA pipetting*	• That one test-tube at time is opened avoiding spilling the contents • That any time the tips has been changed
		Contamination of the Reagents prepared in the session	• That the reagents dispensation has been monitored • That any time the tips has been changed
		Contamination of the AREA designed for the extraction/preparation of the amplification reaftions.	• That the surfaces and tools are cleaned according how described in section F.
		Excess of Extracted RNA in the reaction	• That the concentration of extracted RNA added to Reaction tube has been correctly quantified • Do not exceed the concentration of 40 ng/ul (a total of 1 ug RNA) in the Reverse Transcription
SAMPLE unknown	FALSE NEGATIVE	*Excess of cDNA or Reverse Transcription reagents in the amplification*	• That has been avoided adding an exceeding quantity of Reverse Transcription reaction product into the amplification reaction
		Error during dispensation	• Tha the dispensation has been carefully done
		Loss of activity of the enzymes	• That the tubes has been kept on Ice
		Error in the Thermal Profile setting	• That the Thermal profile set on the thermal cycler has been checked before the experiment starting
		Degradation of the extracted Nucleic Acid	• That DNase and RNase-Free plastic has been used • That all the Good Laboratory Procedures for RNA handling has been strictly followed
		Bad quality of RNA extraction	• Repeat the Extraction step with a new sample

If the results of the test match the *CORRECT RESULT* requirements stated above, proceed to the amplification step following the specific protocol of DRNA.CE kit (Dia.Pro srl)

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.

O. PERFORMANCES

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

P. BIBLIOGRAPHY

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

5. Symbols

LEGENDA			
REF	Product code		Storage temperature
IVD	In Vitro Diagnostic Device		See use instructions
LOT	Lot number		Manufacturer
	Expiry date		Number of tests
CE	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.

CE

DISTRIBUTOR

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MANUFACTURER

Dia.Pro -Diagnostic Bioprobes Srl

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