











HDV 255/500 IC

Key to symbols used

	List Number		Store at 2-8°C/-20°C
	For Research Use Only		Caution!
	Lot Number		Version
	Expiration Date		Consult instructions for use
	Contains reagents		Manufacturer

NAME

HDV 255/500 IC

INTENDED USE

kit **HDV 255/500 IC** is intended for qualitative detection of *HDV RNA* by reverse transcription (RT) and nucleic acid amplification

PRINCIPLE OF ASSAY

kit **HDV 255/500 IC** is based on four major processes: isolation of *HDV RNA* from specimens, reverse transcription of the RNA, amplification of the cDNA and detection of the amplified products on agarose gel. The kit contains the Internal Control which may be used in the isolation procedure and serves as an amplification control for each individually processed specimen and to identify possible inhibition.

MATERIALS PROVIDED

Part N° 1 – “**Reverta-L**”: reverse transcription of the RNA

Part N° 2 – “**HDV 50R**”: amplification kit;

Part N° 1 – “**Reverta-L**”:

- **RT-G-mix-1**, 5 x 0,01 ml;
- RT-mix, 5 x 0,125 ml;
- Reverse transcriptase (M-MLV), 0,03 ml;
- TE-buffer, 1,2 ml.

Contains reagents for 60 tests

Part N° 2 – “**HDV 50R**”:

- PCR-mix-1, 55 ready-to-use single-dose test tubes
- PCR-mix-2, 0,6 mL
- Mineral Oil, 2,0 ml;
- Negative Control C-*, 1,6 mL
- **HDV C+ Rec Fag****, 5 x 0,03 mL
- **HDV IC Rec Fag*****, 5 x 0,06 mL
- **HDV cDNA (C+)**, 0,2 mL
- DNA-buffer (C-), 0,5 mL

Contains reagents for 55 tests.

*must be used in the isolation procedure as Negative Control of Extraction;

**must be used in the isolation procedure as Positive Control of Extraction (add to the tube labeled *C_{pos}* 10 µl of *HDV C+ Rec Fag*)

***add 5 µl of Internal Control during the RNA isolation procedure directly to the sample/lysis mixture (see Ribo-Sorb (REF K-2-1) protocol)

MATERIALS REQUIRED BUT NOT PROVIDED

- Thermalcycler
- Workstation
- Pipettors (capacity 0,5-10 µl; 5-40 µl) with aerosol barrier
- Tube racks

Reagents non provided

- RNA extraction kit (recommended nucleic acid extraction kit: Ribo-Sorb (Sacace, REF K-2-1) o column extraction Ribo Virus (Sacace, REF K-2/C)
- Detection agarose kit

WARNINGS AND PRECAUTIONS

1. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
2. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
3. Do not use a kit after its expiration date.
4. Dispose off all specimens and unused reagents in according with local regulation.
5. Biosafety Level 2 should be used for materials that contain or are suspected of containing infectious agents.
6. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
7. Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
8. Material Safety Data Sheets (MSDS) are available on request.
9. This kit is designed for use with “Ribo-Sorb” extraction kit. It is the user’s responsibility if other kits than “Ribo-Sorb” are used to perform this RNA extraction.
10. Use of this product should be limited to personnel trained in the techniques of DNA amplification.
11. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step

STORAGE INSTRUCTIONS

HDV 255/500 IC Test must be stored at 2-8°C. Part N° 1 – “Reverta-R” must be stored at -20°C. The kit can be shipped at 2-8°C for 3-4 days but should be stored at 2-8°C and -20°C immediately on receipt.

STABILITY

HDV 255/500 IC Test is stable up to the expiration date indicated on the kit label. The product will maintain performance through the control date printed on the label. Exposure to light, heat or humidity may affect the shelf life of some of the kit components and should be avoided. Repeated thawing and freezing of these reagents should be avoided, as this may reduce the sensitivity. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

HDV 255/500 IC Test can analyze RNA extracted with **Ribo-Sorb** (REF K-2-1) o Ribo Virus (Sacace, REF K-2/C) from:

- plasma collected blood in ACD or EDTA tubes;
- Specimens can be stored at +2-8°C for no longer than 12 hours, or frozen at -20°C to -80°C.
Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

RT AND AMPLIFICATION

Reverse Transcription:

- 1) Prepare Reaction Mix: for 12 reactions, add **5,0 µl RT-G-mix-1** into the tube containing **RT-mix** and vortex for at least 5-10 seconds, centrifuge briefly. This mix is stable for 1 month at -20°C. Add **6 µl M-MLV** into the tube with Reagent Mix, mix by pipetting, vortex for 3 sec, centrifuge for 5-7 sec (must be used immediately after the preparation).
(If it is necessary to test less than 12 samples add for each sample (N) in the new sterile tube **10*N µl of RT-G-mix-1 with RT-mix and 0,5*N µl of M-MLV**).
- 2) Add **10 of Reaction Mix** into each sample tube.
- 3) Pipette **10 µl RNA** samples to the appropriate tube. (If the Ribo-Sorb isolation kit is used as a RNA extraction kit, re-centrifuge all the tubes with extracted RNA for 2 min at maximum speed (12000-16000 g) and take carefully supernatant. N.B. don't disturb the pellet, sorbent inhibit reaction). Carefully mix by pipetting.
- 4) Place tubes into thermalcycler and incubate at 37°C for 30 minutes.
- 5) Dilute 1: 2 each obtained cDNA sample with TE-buffer (add 20 µl TE-buffer to each tube).
cDNA specimens could be stored at -20°C for a week or at -70°C during a year.

Amplification:

- 1) Prepare required quantity of **PCR-mix-1** tubes, including two additional tubes - one for **Negative Control of Amplification** and one for **Positive Control of Amplification**.
- 2) Add to the each tube **10 µl PCR-mix-2**.
- 3) Add to appropriate tube **10 µl cDNA** sample obtained after reverse transcription step.
- 4) Add **10 µl DNA-buffer** to the tube for **Negative Control of Amplification**.
- 5) Add **10 µl HDV cDNA (C+)** to the tube for **Positive Control of Amplification**.
- 6) Close PCR-mix-1 tubes and transfer them into the thermalcycler only when temp. reaches 95°C and start the following program:

Thermocyclers with block temperature adjustment: “PTC-100”(MJ Research) BioRad, Biometra				Thermocyclers with active temperature adjustment. PE 2700, Gradient Palm Cycler and other.		
Step	t°C	Time	Cycles	t°C	Time	Cycles
1	95°C	Pause		95°C	Pause	
2	95°C	5 min	1	95°C	5 min	1
3	95°C	1 min	42	95°C	30 sec	42
	67°C	1 min		67°C	30 sec	
	72°C	1 min		72°C	30 sec	
4	72°C	1 min	1	72°C	1 min	1
5	10°C	Storage		10°C	Storage	

RESULTS ANALYSIS

Analysis of PCR results is based on the presence or absence of specific bands of amplified DNA in Agarose gel (2%). The length of specific amplified DNA fragments is:

- **HDV – 255 bp**
- **Internal Control (IC) – 500 bp**

RESULTS INTERPRETATION

Table 2. Results for controls

Control	Which step of test is controlled	Specific bands in the gel 255 bp	Specific bands in the gel 500 bp	Interpretation
NCS	RNA isolation	No	Yes	Valid result
DNA-buffer	Amplification	No	No	Valid result
HDV cDNA (+)	Amplification	Yes	No	Valid result

- Ensure that the Negative Controls (NEG CONTROL and DNA-buffer) values for the run are valid. If the run is invalid (contamination – detection of the specific band or defective extraction/amplification – absence in NEG CONTROL of the band corresponding to Internal Control), the entire test protocol (sample preparation, amplification and detection) should be repeated. Discard any reagents that may be suspect.
- The sample is considered to be positive for **HDV RNA** if the band of 255 bp is observed on agarose gel.

PERFORMANCE CHARACTERISTICS

Analytical specificity

The analytical specificity of the primers and probes was validated with negative samples. They did not generate any signal with the specific **HDV** primers and probes. The specificity of the kit **HDV 255/500 IC** was 100%. The potential cross-reactivity of the kit **HDV 255/500 IC** was tested against the group control. It was not observed any cross-reactivity with other pathogens.

Analytical sensitivity

The kit **HDV 255/500 IC** allows to detect **HDV RNA** in 100% of the tests with a sensitivity of not less than 2500 copies/ml. The detection was carried out on the control standard and its dilutions by negative sample.

Target region: gene coding Dag



Sacace Biotechnologies Srl
18 San Carlo str., 81100 Caserta, Italia