



BIOTOOLS

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BIOHCV Kit

***Kit for Hepatitis C Virus RNA detection (HCV)
in human clinical samples***

Instructions for Use

Ref. 90.063

***PLEASE READ THE INSTRUCTIONS FOR USE THOROUGHLY BEFORE USING THE KIT,
ESPECIALLY IF YOU ARE NOT FAMILIAR WITH THE PROTOCOL***

BIOHCV Kit

For research use only (RUO)
Not for use in diagnostic procedures
Test results may be used for preliminary analysis only

Some of the applications which may be performed with this product may be in certain countries under an applicable patent. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application. Biotools does not encourage the unlicensed use of patented applications.

PLEASE CHECK INTEGRITY OF KIT AND REAGENTS BEFORE USE. DETERIORATED KITS MAY CAUSE EQUIVOCAL RESULTS.

1. INTENDED USE

The BIOHCV Kit is a method for the qualitative determination of Hepatitis C Virus (HCV) in clinical samples by reverse transcription followed by DNA amplification in clinical samples. The kit reverse transcribes viral RNA into cDNA, which is then amplified using specific set of primers. The kit has been tested with samples from European, Asian, African and Middle East origin.

The BIOHCV Kit is to be used with the following clinical samples:

- Blood samples stored in EDTA. Use of blood samples with heparine or citric acid has not been tested, and therefore, the kit may not render the expected results.
- Serum / plasma samples stored in heparine. Use of serum / plasma samples in other conditions has not been tested, and therefore, the kit may not render the expected results.
- PBMC samples stored at or below -20°C . For prolonged storages of PBMC storage at -80°C or in liquid nitrogen is recommended.
- Liver biopsies. For the use of the Kit with biopsies do not treat the tissue with acetic acid or iodide Paraffin-embedded tissues can be used provided the employed fixative reagents do not degrade or interact with nucleic acids and deparaffination step is performed.

2. SUMMARY AND EXPLANATION OF THE TEST

Hepatitis C virus is the etiological agent of most post-transfusion non-A, non-B hepatitis cases¹. HCV is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Around 170 million persons are chronically infected with HCV and 3 to 4 million persons are newly infected each year².

Traditional diagnosis methods are based on serology, using immunological methods. Diagnosis based on antibodies does not generally differentiate between past and present infections. Viral antigen-based diagnosis is widely used as screening method. However, patients at early stages of the disease, or infected by mutant serotypes of the virus, can render false negative results, due to the lack of detectable antigen in the analysed sample³.

Nucleic acid-based techniques allow early detection of the viral presence in the patient, as well as detection of most mutant serotypes⁴. They can detect viral presence in the window period, or at minute levels of virus, so that they can be used for early diagnosis as well as treatment monitoring⁵.

The BIOHCV Kit allows the detection of hepatitis C virus RNA in a wide variety of clinical samples (whole blood, serum, plasma, PBMCs, hepatic tissues, etc.).

3. PRINCIPLES OF THE PROCEDURE

The BIOHCV Kit is a test for qualitative analysis of HCV, using a reverse transcription / DNA amplification technique for direct detection of viral nucleic acids sequences through agarose electrophoresis and ethidium bromide staining.

Viral RNA present in positive samples is specifically reverse transcribed into cDNA by using a specific primer (P1), which hybridise with homologous sequences in the viral genome. The obtained cDNA is then

¹ Bukh *et al.* (1992). Proc Natl Acad Sci USA, 89: 4942-4946.

² World Health Organisation Fact Sheet 164 (2000).

³ Young *et al.* (1993). J Clin Microbiol, 31: 882-886.

⁴ Bukh *et al.* (1992). Proc Natl Acad Sci USA, 89: 187-191.

⁵ Schreiber *et al.* (1996). New Engl J Med, 334: 1685-1690.

amplified in two reactions, being the second amplification a nested reaction which achieves maximum sensitivity and specificity of the detection.

The kit consists on the following steps: first, a reverse transcription is performed so that viral RNA is converted into cDNA using a specific primer P1; second the obtained cDNA is then amplified exponentially using the pair of primers P1/P2, which hybridises with sequences common to all HCV isolates known and tested so far; finally a second pair of primers P3/P4 hybridises with the amplification product obtained in the previous reaction (nested amplification reaction), allowing an increase in the sensitivity and specificity of the detection reaction.

For analysing the obtained results an agarose electrophoresis must be performed, followed by ethidium bromide staining. The first pair of primers renders a non-detectable product, except in samples with a high viraemia which render a 350 bp band. The final amplification product (nested amplification results) must render a 260 bp band.

A) Sample Preparation

The BIOHCV Kit is used with RNA purified from clinical samples. For viral RNA purification, **BIOTOOLS recommends the use of Speedtools RNA Virus Extraction Kit** (Ref. 21.141/2), though other methods guaranteeing $A_{260/280}=1.8 - 2.0$, 50-100 ng/ μ l concentration and the absence of amplification inhibitors can also be employed.

Check the quality and suitability of the purified RNA for reverse transcription / amplification reactions, for example, by performing control amplifications in parallel. For further information, please contact our Technical Dept. (info@biotools.eu).

B) Reverse Transcription, Amplification and Detection

Target selection

Selection of the target HCV sequences has been based on the study of highly conserved regions in the HCV genome (5' UTR region). Selected regions have a high degree of conservation between the tested HCV variants.

Reverse transcription and amplification

Reverse transcription of viral RNA is performed with a modified thermostable DNA polymerase from *Thermus spp.* (RetroTools® Polymerase). In the presence of manganese, and with the suitable salt and ionic strength conditions, the enzyme shows reverse transcriptase activity using as anchor a primer and a RNA molecule as template. On the other hand, substitution of manganese by magnesium allows the enzyme to switch its activity from reverse transcriptase to polymerase, therefore achieving both reactions in a single tube, which facilitates Kit manipulation.

RNA purified from the sample to be analysed is added to the reaction mixture made with the reagents provided by the Kit. Reaction mixture with the RNA, is incubated at different temperatures, in order to allow the hybridisation of the primer P1 to the template. The DNA polymerase in the presence of triphosphate deoxynucleotides, extends the primer by reverse transcription, and forms a cDNA strand complementary to the template RNA. The cDNA is then used as template for the first amplification reaction with the primers P1/P2. The mixture is then incubated at different temperatures, the DNA polymerase in presence of Mg and triphosphate deoxynucleotides acts as a polymerase amplifying the target cDNA sequence. Afterwards a nested amplification is performed using the product of the first amplification and the primers P3/P4. Cyclic repetition of this process results in exponential amplification of the sequence originally present in the sample included between the primer pairs.

Detection

Detection of amplified products is performed by agarose gel electrophoresis followed by ethidium bromide staining.

NOTE

Ethidium bromide is a highly mutagenic intercalating agent. We recommend the use of gloves and the maximum caution in handling.

The presence of HCV in the analysed samples is indicated by a band of approximately 260 bp. For samples with a high viraemia a band of 350 bp may appear after the first amplification, this band does not interfere with the final result.

4. REAGENTS

The Kit contains reagents in liquid format for performance of 48 amplification reactions (Ref. 90.062). To minimize the risk of contamination and facilitate the use of the kit several times the Kit is presented in two set of 24 reactions. Sample Kit (Ref. 90.061) contains one set of 24 reactions. **Store all Kit vials at -15±8°C, except HCV Buffer which must be stored at room temperature.** Thaw and handle reagents on ice. Do not freeze/thaw Kit vials repeatedly. In case of frequent use, we recommend the aliquoting of the vial contents.

- **RT Mixture:** Vial: 1 x 270 µl
A Tris-HCl solution, containing <10 % glycerol, KCl, <0.001 % dATP, dCTP, dGTP, dTTP and primers P1/P2. The RT mixture vial contains all the reagents, except MnCl₂ and RetroTools® Polymerase, in the optimal concentration, to allow an effective reverse transcription of the viral RNA.
- **5 mM MnCl₂ Solution:** Vial: 1 x 300 µl
Mix well before use.
- **RetroTools® Polymerase:** Vial: 1 x 150 µl
Add to reaction mixes shortly before introduction in the thermal cycler.
- **HCV Buffer:** Vial: 1 x 130 µl
A combination of inorganic factors to enhance reverse transcription efficiency. **Store at room temperature (maximum 25°C).**
- **Amplification Mixture:** Three vials: 3 x 1400 µl
Solution containing all the reagents needed (MgCl₂) for the amplification of the cDNA obtained in the previous reaction.
- **Nested Mixture:** Three vials: 3 x 1575 µl
A Tris-HCl solution, containing <10 % glycerol, KCl, <0.001 % dATP, dCTP, dGTP, dTTP and primers P3/P4. The Nested mixture vial contain all the reagents, in the optimal concentration, to allow an effective amplification of the DNA obtained after the first amplification.
- **Positive Control:** Vial: 1 x 320 µl
Non-infective positive control of HCV. It consists of amplified RNA product, containing a generic sequence from HCV, flanked by the primers. To be analysed in a separate amplification reaction. It can also be used as intra-tube control.

5. INSTRUCTIONS FOR USE

NOTE

Thaw and keep reagents on ice while in use. For optimal results keep reaction mixtures and vials in refrigerated conditions until their introduction into the thermal cycler.

To avoid RNA degradation, use sterile bidistilled DEPC treated water in all the process, and keep RNA samples on ice. To avoid RNase contamination during handling, use latex examination gloves.

A. REVERSE TRANSCRIPTION REACTION

Proceed to Reagent Preparation Area in a laminar flow cabinet

1.- Final reaction volume of the reverse transcription reaction is 10 µl. Prepare the **Reverse Transcription Mixture** following the table bellow (Table 1) in a 1.5 ml vial, according to the number of reactions to be performed (Reverse Transcription Mixture = RT Mixture + MnCl₂ + RetroTools® Polymerase + HCV Buffer). For each round of analysis include at least one positive control and one negative control. To ensure sufficient volume for all reactions, prepare the Reverse Transcription Mixture for n+1 reactions.

Table 1. Reverse Transcription Mixture

REAGENT	For 1 Reaction
RT Mixture	2.55 µl
5 mM MnCl ₂ Solution	2 µl
RetroTools® Polymerase	1.25 µl
HCV Buffer	1.2 µl

2.- Aliquot **7 µl** of the **Reverse Transcription Mixture** in each reaction vial.

Remove vials from laminar flow cabinet and proceed to RNA Purification Area. Never introduce RNA from samples or positive controls in the laminar flow cabinet at the reagent preparation area. The reverse transcription reaction must start in the next 10 minutes after adding purified RNA from samples and controls to the reaction mixture.

3.- Add **50-100 ng RNA** from the purified samples to each reaction vial. **Complete up to 10 µl final volume with DEPC sterile bidistilled water.** Maximum sample volume: 3 µl. Minimum sample volume: 1 µl.

NOTE

Clinical samples, depending on their nature and viraemia levels, may have different RNA concentrations, and therefore, amount of template to be added to the amplification reaction is expressed in ng rather than indicating sample volume. Quantity and purity of template must be calculated, e.g. by measuring A_{260/280} values.

4.- **Positive Controls** should be prepared by adding **3 µl of Positive Control vial**. **Negative Controls** should be prepared with **3 µl of DEPC sterile bidistilled water**.

Proceed to the Amplification Area

5.- Close vials and place them in the thermal cycler. Store remaining of all reagents (except HCV Buffer which is stored at room temperature) at **-15±8°C**.

6.- Perform the reverse transcription using the following program:

Reverse Transcription Program	
INITIAL DENATURING	85°C / 1 min
REVERSE TRANSCRIPTION	60°C / 30 min
	4 °C / ∞

NOTE

This protocol has been adapted for Eppendorf, MJ Research and Applied Biosystems GeneAmp™ thermal cyclers. For other thermal cyclers, optimisation of reaction parameters may be necessary. For any question, please contact our Technical Dept. (info@biotools.eu).

7.- -The reverse transcription and the first amplification must be performed on the same day. Therefore **do not freeze the reaction vials after completion of the reverse transcription, keep them on ice and proceed to the amplification step.**

B. FIRST AMPLIFICATION REACTION

Proceed to Reagent Preparation Area in a laminar flow cabinet

8.- Final reaction volume of the first amplification reaction is 50 µl. Add 40 µl of the Amplification Mixture Vial provided by the Kit to each of the reaction vials where reverse transcription was performed.

Proceed to the Amplification Area

9.- Perform the following program for the first amplification reaction:

First Amplification Program	
INITIAL DENATURING	94°C / 2 min
CYCLIC AMPLIFICATION	94°C / 30 sec
	55°C / 30 sec
	72°C / 30 sec
NUMBER OF CYCLES	40
FINAL ELONGATION	72°C / 5 min
	4 °C / ∞

NOTE

This protocol has been adapted for Eppendorf, MJ Research and Applied Biosystems GeneAmp™ thermal cyclers. For other thermal cyclers, optimisation of reaction parameters may be necessary. For any question, please contact our Technical Dept. (info@biotools.eu).

10.- Store amplified reaction products at 2-8°C. Store at room temperature only if the next amplification is performed within 1-2 hours.

C. NESTED AMPLIFICATION REACTION

Perform this process in a laminar flow cabinet at the Pre-amplification Area

11.- Prepare the **Nested Amplification Mixture** following the table below (Table 2) in a 1.5 ml vial, according to the number of reactions to be performed (Nested Amplification Mixture = Nested Mixture + RetroTools® Polymerase). For each round of analysis include at least one positive control and one negative control. To ensure sufficient volume for all reactions, prepare the Nested Amplification Mixture for n+1 reactions.

Table 2. Nested Amplification Mixture

REAGENT	For 1 Reaction
Nested Mixture	44.8 µl
RetroTools® Polymerase	0.2 µl

12.- Take a new set of amplification vials and aliquot 45 µl of the Nested Amplification Mixture in each new amplification vial.

Remove vials from laminar flow cabinet and proceed to the DNA Purification Area

13.- Add 5 µl from the corresponding product obtained in the previous amplification.

14.- Close vials and place them in the thermal cycler. Store remaining of all reagents at temperatures under -15±8°C.

15.- Perform the following program for the nested amplification:

Nested Amplification Program	
INITIAL DENATURING	94°C / 2 min
CYCLIC AMPLIFICATION	94°C / 30 sec
	55°C / 30 sec
	72°C / 30 sec
NUMBER OF CYCLES	40
FINAL ELONGATION	72°C / 5 min
	4 °C / ∞

NOTE

This protocol has been adapted for Eppendorf, MJ Research and Applied Biosystems GeneAmp™ thermal cyclers. For other thermal cyclers, optimisation of reaction parameters may be necessary. For any question, please contact our Technical Dept. (info@biotools.eu).

16.- Store nested amplification products at 2-8°C. Store at room temperature only if electrophoresis is performed within 1-2 hours.

6. INTERPRETATION OF RESULTS

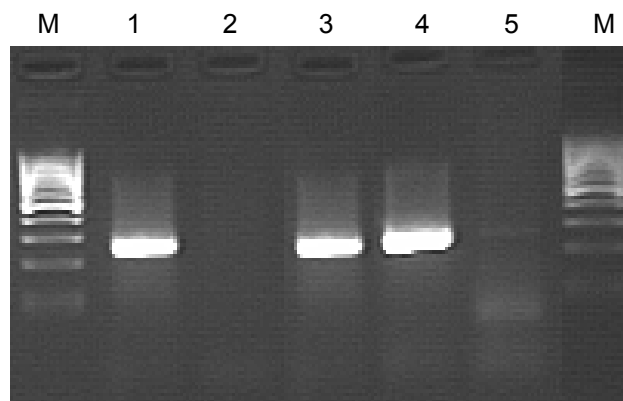
The analysis of the amplification products is performed by horizontal electrophoresis in low EEO-agarose gels (e.g. MB Agarose, Ref. 20.011). Band visualisation is improved in 1.5-2 % gels using TAE 1X or TBE 0.5X as running buffers. It is recommended to add ethidium bromide in the agarose gel for a better resolution and visualisation.

NOTE

Ethidium bromide is a highly mutagenic intercalating agent. Use of gloves and maximum caution is recommended on handling this reagent.

Samples containing HCV will render a band of approximately 260 bp. For samples with a high viraemia, a band of approximately 350 bp may appear after the first amplification reaction. This band does not interfere with the results.

Figura 1. Screening of serum samples from patients affected by HCV chronic hepatopathy. Patients having high viraemia levels (Lane 1, Lane 3 and Lane 4); patient with low viraemia level (Lane 5); negative control (Lane 2); M: 100 bp Ladder (Ref. 31.006).



7. MATERIALS REQUIRED BUT NOT PROVIDED

NOTE

For all equipments, regular maintenance and calibration is necessary. Follow manufacturer's instructions, and check working parameters regularly, specially for thermal cyclers and pipettes. Maintenance and calibration of instruments allows its correct functioning, and helps detecting problems that may render an incorrect analysis result.

Pre-amplification area (RNA/DNA purification and reagent preparation areas)

- Equipment, reagents and disposable material necessary for RNA purification (depending on the method, follow manufacturer's instructions)
- Timer
- Automatic pipettes⁶ (10, 20 and 200 µl), filter or positive displacement tips, RNase-free⁷
- Disposable examination gloves, powder-free
- DEPC-sterile bidistilled water
- Screw cap polypropylene tubes, 1.5 ml capacity, non siliconised, conical, sterile, RNase-free. It is recommended to use screw cap tubes, in order to avoid the potential contamination of samples and controls.
- Racks for 1.5 ml vials
- Containers for disposal of potentially-infectious material
- Disposable filter paper for working surface, cleaning paper for accidental spills
- Termini-DNA-Tor⁸ or equivalent, in order to remove DNA from working surfaces

Amplification area

- Thermal cycler: Eppendorf MasterCycler™ Personal, MJ Research MiniCycler™ or Applied Biosystems GeneAmp™ 2700. Use of this kit in other equipments has not been tested. For further information, contact our Technical Dept. (info@biotools.eu)
- Laminar flow cabinet
- Racks for reaction vials
- Reaction vials (0.2 ml, thin-walled)
- DEPC-sterile bidistilled water (Ref. 20.033 or equivalent)
- Automatic pipettes (10, 20 and 200 µl), filter or positive displacement tips, RNase-free
- Disposable examination gloves, powder-free
- Containers for disposal of potentially-infectious material
- Disposable filter paper for working surface, cleaning paper for accidental spills
- Termini-DNA-Tor or equivalent, in order to remove DNA from working surfaces

Post-amplification area

- Electrophoresis power supplies and tanks
- Gel Documentation system
- UV transilluminator
- Ethidium bromide
- Low EEO agarose (Ref. 20.011) or equivalent
- TAE or TBE
- DNA Ladder ranging between 150 to 700 bp (Ref. 31.006) or equivalent
- Electrophoresis loading buffer
- Automatic pipettes (10, 20 and 200 µl), filter or positive displacement tips, RNase-free
- Disposable examination gloves, powder-free
- Protective mask / goggles for UV
- Microwave

8. WARNINGS AND PRECAUTIONS

Following is a list of warning and precautions. For a complete information, we recommend to read the Material Safety Data Sheet (MSDS), available in our webpage (www.biotools.eu), or by request to our Technical Dept. (info@biotools.eu).

A. Research Use Only.

⁶ Precision of automatic pipettes must be in the range of 3 % of the indicated volume. If necessary, calibrate and check regularly, following manufacturer's instructions. It is recommended to use RNase-free filter tips and positive displacement tips, in order to avoid cross contamination between samples and amplicons.

⁷ It is recommended to use different sets of pipettes for each reaction step (pre-amplification, amplification, post-amplification), in order to avoid contaminations that may render false positive results.

⁸ Available in Biotools' catalogue (Ref. No. 22.001, 22.002).

- B. This test must be used with samples collected, handled and stored as indicated in the corresponding chapter. Efficiency of the test in other samples has not been tested.
- C. The kit detects HCV RNA, and has been tested with different samples from different geographical origins (Europe, Middle East, Africa, Asia). Samples from other geographical areas have not been tested at the company laboratories. Some HCV isolates may present mutations at the primers' annealing sites, therefore affecting quality of the result. This applies for samples from geographical origins not tested in our laboratories, or for samples from the same geographical origin as those tested in our laboratories, but with mutations at the primers' annealing sites. The kit only detects presence/absence of HCV RNA, and is not intended for diagnosis or for quantification of viral loads.
- D. The correct handling and storage conditions of RNA samples is a critical step for optimal results in reverse transcription experiments. Be careful during RNA sample handling, and use sterile products, and latex examination gloves. Store RNA samples at -80°C . If this is not possible, store samples at temperatures below -18°C .
- E. Handle all samples and discarded material as infectious or potentially infectious.
- F. Use powder-free examination gloves while handling reagents or samples, as well as lab coat. Wash hands thoroughly after performing the test.
- G. Open and close reagent vials carefully. Observe temperature and light exposure instructions. After use, close vials and store at indicated temperatures.
- H. All materials used with the kit, including reagents and samples, must be discarded as to inactivate all possible infectious agents
 1. **Solids:** autoclave.
 2. **Liquids:** add sodium hypochloride⁹ at a final concentration of 1 %, and incubate 30 minutes at room temperature before discarding any material.
- I. Spills: wash spills with a 5 % solution of sodium hypochloride. Cover surface with absorbent material, saturated with a 5 % solution of sodium hypochloride. Let at least for 10 minutes. In order to avoid fume exposure, a plastic or glass cover can be used. All materials used for washing spills must be treated as infectious or potentially infectious material.
- J. Do not use product after expiry or best before date.
- K. Kit components have been tested as a whole. **Do not interchange components** with other kits, or components from different lots
- L. Nucleic acids are very sensitive to degradation by nucleases. Nucleases are present in human skin and surfaces that have been in contact with humans. Wash with Termi-DNA-Tor and cover working surfaces with suitable paper. Use powder-free examination gloves throughout the whole process.
- M. Extreme care must be taken when aliquoting the different volumes in each reaction step. Mix well after addition of each reagent, unless otherwise noted. Read instructions for use of automatic pipettes.
- N. Do not pipette by mouth.
- O. Packaging material included with the kit is resistant to the indicated storage conditions. Storage at different conditions can cause breakage of the material, and possible contamination of kit contents.
- P. Plastic material included with the kit is resistant in the normal conditions of use. Use of plastic material in extreme conditions may cause its breakage, and therefore, impossibility to use the kit.
- Q. Kit reagents, once used, must be discarded. Reagents cannot be reused once they have been used for the analysis of clinical samples, as this may cause false positive or false negative results.
- R. Laboratory workflow must be unidirectional, from pre-amplification area to post-amplification area. Specific equipment for each working area must be used, in order to avoid cross contaminations. Equipment used for amplification must remain in this area at all times.

9. STORAGE AND HANDLING INSTRUCTIONS

1. After reception, store the different reagents at the indicated temperatures (at $-15\pm 8^{\circ}\text{C}$); except HCV Buffer which must be stored at room temperature). Use non frost-free freezers. Also, for frequent use (more than 1 time a week), aliquot the contents of the vials in different tubes, in order to avoid repeated freeze/thaw cycles.
2. Do not use the kit after expiry date. The closed kit is stable until the indicated date, if storage instructions are correctly followed. Do not mix reagents from other kits and/or other lots. If trace amounts of reagents remain, they must be discarded.

10. SAMPLE COLLECTION, TRANSPORT AND STORAGE

The different clinical samples recommended for use with the BIOHCV Kit are indicated at the beginning of this Manual. Samples taken with other methods or transported according to different specifications have not been tested for use with the kit. Biopsy samples can be used with the Kit but before applying acetic acid or iodide to the sample.

⁹ Commercial bleach usually contains sodium hypochloride at a concentration of 5 %. Bleach can be used, after performing the necessary calculations in order to achieve the indicated concentration.

Samples stored in EDTA

Samples should be stored at 2-8°C and shipped at this temperature to the clinical analysis laboratory. For prolonged storages, we recommend freezing. Once in the laboratory, they can be stored at 2-8°C if analysis is to be performed in one week. If the analysis is to be performed later, store samples at -15±8°C or -70°C. Samples must be kept away from heat sources and preserved of environmental humidity. Bacterial growth must be avoided and RNA integrity must be kept.

Samples stored in heparine

Samples may be stored up to three days at 2-8°C, and shipped with refrigeration to the clinical analysis laboratory. For prolonged storages, we recommend temperatures of -20°C. Once in the laboratory, they should be stored at -15±8°C or -70°C. Samples must be kept away from heat sources and preserved of environmental humidity. Bacterial growth must be avoided and RNA integrity must be kept.

Liver biopsies

Fresh biopsies of up to 5 mm must be used. Biopsy must be immediately placed in a preservative medium and stored at -15±8°C. Biopsies can be shipped by express courier (24 hours maximum) at a maximum temperature of 25°C, and stored at -15±8°C in the laboratory until performance of the test. Biopsies with a diametre under 2 mm must not be used.

Use of paraffin-embedded tissues is possible provided that tissue fixation method do not degrade nucleic acids and purification of RNA is performed with methods specific for this kind of sample.

PBMCs

PBMCs should be stored and shipped at -15±8°C. For prolonged storages (more than one week), storage at -80°C or in liquid nitrogen is recommended.

In order to avoid accidents or accidental opening of the sample container, it is recommended to seal its closure with Parafilm® or equivalent before freezing.

Sample shipment must comply with local, national and international regulations for transport of etiological agents.

11. QUALITY CONTROL

It is recommended that at least one (1) Positive Control and one (1) Negative Control be run each time the test is performed. As with any new laboratory procedure, novel users should consider performing additional controls (both positive and negative) until a high degree of confidence is reached.

The Positive Control must render a 260 bp band. Vials containing negative control (DEPC sterile bidistilled water) must render no bands. Any analysis not fulfilling any of these results must be completely invalidated and discarded. It is necessary to repeat the process from its beginning, including RNA purification, processing other aliquot of the original sample. A failure of instruments during the test, indicated by error messages, also means that the test has not been valid. Repeat all the procedure for each sample from the reverse transcription step.

For laboratories requiring an external control, this must contain a defined number of copies of the target sequence for HCV, and the viraemia levels in this control must be a multiply of the limit value of the testing procedure (e.g. WHO standards for HCV RNA).

12. PROCEDURAL PRECAUTIONS

1. Laboratory workflow must be unidirectional, from pre-amplification area to post-amplification area. Pre-amplification tasks must be initiated with the preparation of the reagents and sample purification. Equipments, materials and reagents must be dedicated and they must not be used for other activities or be transferred from one to another area. Gloves must be worn in each area, and must be discarded before proceeding to the next area. Equipments and materials used for setting-up of reactions must not be used for other activities, or for pipetting or processing amplified DNA or other RNA sources.
2. As with any analytical procedure, it is fundamental to use a good laboratory practice to obtain good results with this technique. Due to the high analytical sensitivity of the test, extreme care must be taken in order to keep the purity of all kit reagents and all reaction mixes. All reagents must be carefully checked in order to ascertain their purity. Discard all suspect reagents.

13. PROCEDURAL LIMITATIONS

1. Research Use Only.
2. Instructions must be followed in order to obtain correct results. Should the user have any questions, please contact our Technical Dept. (info@biotools.eu).
3. This test has been validated for use with the reagents provided by the kit. The use of other amplification methods, or the use of equipment not fulfilling the specifications, may render equivocal results. User is responsible for validating the modifications for this test, in any of the indicated parameters.
4. This test has been validated with samples collected and shipped as per the "Sample Collection" chapter. Any modification has not been validated, and therefore, obtained results may not be correct.
5. Detection of HCV RNA depends on the number of viral particles present in the sample, and can be affected by sample collection methods, patient-related factors (e.g. age, symptoms), or for infection stage and sample size.
6. False negative results may be obtained due to polymerase inhibition. It is recommended to perform control reactions to distinguish between inhibition and true negatives.
7. Cross contamination between samples and exogenous RNA/DNA can only be avoided by following good laboratory practice. Instructions in this document must be strictly followed.
8. Use of this product is limited to qualified professional personnel, experienced in RNA purification, reverse transcription and DNA amplification techniques.
9. It is important to pipette the indicated amounts, and mix well after each reagent addition. Check pipettes regularly.

14. WARRANTY

Products are guaranteed to conform to the quality and content indicated on each vial and external labels during their shelf life. BIOTOOLS obligation and purchaser's rights under this warranty are limited to the replacement by BIOTOOLS of any product that is shown defective in fabrication, and that must be returned to BIOTOOLS, freight prepaid, or at BIOTOOLS' option, replacement of the purchasing price.

Any complaint on damaged goods during transport must be directed to the handling or transport agent.

Product for Research Use Only. This product must be used by qualified professionals only. It is the responsibility of the user to ascertain that a given product is adequate for a given application. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits our responsibility to the replacement of the product. No other warranties, of any kind, express or implied, including, without limitation, implicit warranties of commercialisation ability or adequacy for a given purpose, are provided by BIOTOOLS. BIOTOOLS will not be held responsible for any direct, indirect, consequential or incidental damage resulting of the use, misuses, results of the use or inability to use any product.

Manufactured by:

BIOTOOLS, Biotechnological & Medical Laboratories, S.A. has been evaluated and certified to accomplish ISO 9001:2000 requirements for the following activities: Research and development of biotechnology products and manufacture of biotechnology and in vitro products. Valle de Tobalina – 52 – Nave 39, 28021 Madrid – Spain

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Parafilm® is a registered mark of Pechiney Plastic Packaging, Inc.

MasterCycler™ is a trademark of Eppendorf GmbH.

MiniCycler™ is a trademark of MJ Research Inc.

GeneAmp™ is a trademark of Applied Biosystems Inc.

