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სახელმწიფო რეგულირების
სააგენტოს უფროსის
ბატონ გ. თვალავაძეს

დაწესებულების დასახელება: შ. პ. ს. "ჰუმან დიაგნოსტიკ ჯორჯია"
იურიდიულ-სამართლებრივი ფორმა: შეზღუდული პასუხისმგებლობის საზოგადოება

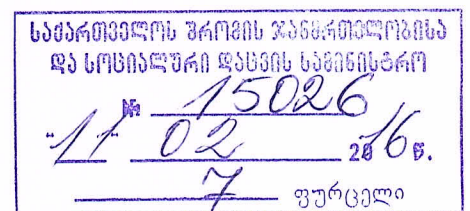
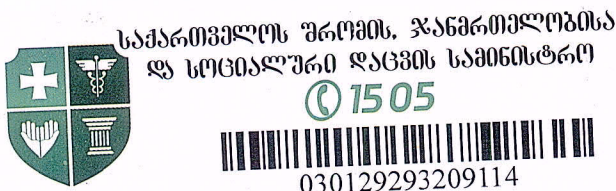
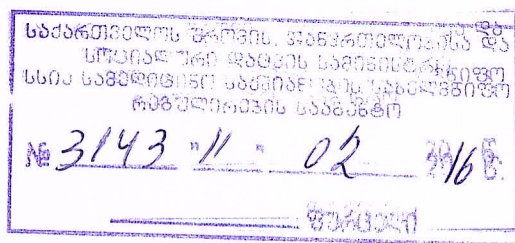
გ ა ნ ც ხ ა დ ე ბ ა

გთხოვთ, დაგვიდასტუროთ, რომ გერმანული კომპანია Human GmbH-ის Real-Time PCR-ის ვირუსული ნუკლეინის მუყეის გამოყოფი კიტი, ვირუსული DNA-ს გამოყოფი კიტი და ვირუსული RNA-ს გამოყოფი კიტი :

1. Viral Nucleic Acid Isolation kit (87300)
 - 1.1 Tubes & Buffers (87301)
 - 1.2 Proteinase K & RNA carrier (87302)
2. Viral DNA Isolation kit (87310)
 - 2.1 Tubes & Buffers (87311)
 - 2.2 Proteinase K & RNA carrier (87312)
3. Viral RNA Isolation kit (87320)
 - 3.1 Tubes & Buffers (87321)
 - 3.2 Proteinase K & RNA carrier (87322)

საქართველოს კანონმდებლობის თანახმად არ ექვემდებარება რეგისტრაციას.

განცხადებას თან ახლავს კიტების აღწერა



VIRAL NUCLEIC ACID ISOLATION KIT

For isolation and purification of viral DNA and RNA from human serum or plasma (EDTA) samples for in vitro diagnostic purposes

Package Size

[REF]	87300	100 Tests	Complete Test Kit
[VD]			

Intended Use

VIRAL NUCLEIC ACID ISOLATION KIT is an in vitro nucleic acid extraction system to isolate nucleic acids from biological materials. It is intended as sample preparation kit to be used in combination with diagnostic kits such as HUMAN REAL-TIME PCR KITS. Viral DNA and RNA are purified from intact cells or cellular structures, which can be further used in sensitive molecular as well as diagnostic downstream applications such as PCR, Real-Time PCR, cloning, RFLP analysis and sequencing. This kit has been validated for use with human serum or human plasma collected in EDTA anticoagulant. VIRAL NUCLEIC ACID ISOLATION KIT (**[REF]** 87300) consists of the 2 components TUBES & BUFFERS (**[REF]** 87301) and PROTEINASE K & RNA CARRIER (**[REF]** 87302). These components will be stored differently (see "Storage and Stability").

Principle of the Test

Isolation of highly-pure viral nucleic acid from serum and plasma samples is essential for clinical diagnosis and molecular research. VIRAL NUCLEIC ACID ISOLATION KIT provides a rapid and simple method for the isolation of viral nucleic acids from virus containing cell-free body fluids. As specifically optimized lysis/washing solutions and silica based spin columns designed to capture exclusively viral nucleic acids are employed, high efficiency and purity is achieved by the end of the isolation process.

250 µl serum or plasma sample are used for a single test. Due to the simple procedure of the kit that requires only basic laboratory equipment, it is possible to isolate viral nucleic acids from various samples simultaneously. The recovered viral nucleic acids can be used directly in downstream applications such as RT-PCR, qRT-PCR, qPCR, viral detection, viral load determination and viral genotyping.

The procedure consists of the following steps: Under highly denaturing conditions lysis and inactivation of nucleases is accomplished in serum and plasma samples. RNA Carrier is added at start serving to enhance the binding affinity of viral nucleic acids to the columns and to decrease the degradation possibility of viral nucleic acids by remaining nucleases. After completion of lysis, the free viral nucleic acid is transferred into columns and the silica gel based membrane captures the viral nucleic acids selectively. In the following consecutive washing steps, contaminants such as proteins, nucleases or inhibitors are efficiently washed away and highly pure viral nucleic acids are eluted.

Reagents and Contents

Components of **[REF]** 87302

[CARRIER]	2 vials	RNA Carrier*
[PROTEINASE]	2 vials	Proteinase K*

Components of **[REF]** 87301

[RL]	2 x 40ml	Solution RL
[W1]	1 x 38ml	Solution W1*
[W2]	1 x 15ml	Solution W2*
[E]	1 x 12ml	Solution E
[COLUMN]	100	Spin Columns
[COL2ML]	700	Collection tubes (2.0 ml)
[COL1.5ML]	100	Collection tube (1.5 ml)

*For preparation of RNA Carrier, Proteinase K and the wash buffers **[W-W1]** and **[W-W2]** see "Notes before Starting" section.

Storage and Stability

The components of TUBES & BUFFERS (**[REF]** 87301) of the VIRAL NUCLEIC ACID ISOLATION KIT should be stored dry at room temperature (15...25°C). RNA Carrier **[CARRIER]** and Proteinase K **[PROTEINASE]** (**[REF]** 87302) should be stored at -20°C immediately upon arrival. Higher storage temperatures should be avoided. Under these conditions, the kit is stable through the expiration date printed on the label. If solutions of the kit are stored at lower temperatures than suggested, in some of them precipitation may occur, thus causing a decrease in the performance of the kit.

Additional Material Recommended but not Supplied with the Kit:

- 96-100 % ethanol (Molecular Biology grade) **[EtOH]**
- Disposal powder-free gloves
- Micropipettes (0.5 µl-1000 µl) with sterile micropipette tips with filters

- Sterile dH₂O
- Microcentrifuge tubes (2.0 ml)
- Vortex mixer
- Benchtop microcentrifuge for 2.0 ml tubes and for PCR strip tubes
- Thermomixer

Warnings and Precautions

All clinical specimens and all resulting waste materials should be treated as potentially infectious; the samples should be prepared in Bio-safety Level 2 area.

The procedure should be preferably performed in four separated areas (i.g. for extraction, PCR setup, sample addition and amplification) to avoid contaminations. All items for the particular procedure should be stored in the area where the procedure is performed and should not be exchanged between these areas. The workflow should be unidirectional, beginning in the extraction area followed by downstream application areas.

Before and after work all surfaces should be disinfected with a freshly prepared solution of 10 % bleach or antiviral agents.

Some of the solutions contain guanidinium salts that form reactive compounds and toxic gases if mixed with bleaching or acidic solutions. Do not add bleach or acidic solutions directly to the sample preparation waste.

Handle all materials containing specimens or controls according to Good Laboratory Practices.

Do not mix reagents with different lot numbers or substitute reagents from other manufacturers.

Do not use a kit after its expiration date.

Store the kit away from any source of contaminating DNA or RNA, especially amplified nucleic acid.

If the VIRAL NUCLEIC ACID ISOLATION KIT substitutes another isolation system, both test systems should be used in parallel for at least two subsequent samples.

Notes before Starting

The amount of recovered DNA/RNA depends strictly on the nuclease contamination of surroundings. Nucleases are very stable enzymes and just need minutes to degrade nucleic acids. Thus decontamination of hands, plastic and glassware contacting the solutions and working space is very important. Through the procedure, disposable latex or vinyl gloves should be worn all the time, RNase/DNase-free disposable tips and microcentrifuge tubes should be used.

Depending on the ambient temperature precipitation may occur in Solution RL **[RL]** during long term storage. Before each use, Solution RL **[RL]** should be checked for precipitates. If precipitation is observed, the solution should be incubated in a 37°C water bath until solution is homogenous again.

Before use, add 800 µl of Solution E **[E]** to lyophilized RNA Carrier tube **[CARRIER]** and 1100 µl of Solution E **[E]** to one Proteinase K tube **[PROTEINASE]**. Mix well by vortexing. The dissolved RNA Carrier solution **[W-CARRIER]** and Proteinase K solutions **[W-PROTEINASE]** should be divided into conveniently sized aliquots and stored at -20°C. The aliquots should not be freeze-thawed more than 3-4 times.

Before use, add 38 ml ethanol (96-100 %) **[EtOH]** to Solution W1 **[W1]** and 60 ml ethanol (96-100 %) **[EtOH]** to Solution W2 **[W2]** to produce the working solutions **[W-W1]** and **[W-W2]**. Solution bottles should be tightly capped between uses to prevent evaporation. The solutions to which ethanol has been added should be kept at room temperature (15...25°C).

Procedure

- Add 20 µl of Proteinase K **[W-PROTEINASE]** and 250 µl of serum or plasma sample into a 2.0 ml tube. If the serum or plasma is frozen, thaw in a 37°C water bath.
- Add 750 µl Solution RL **[RL]** and 15 µl RNA Carrier **[W-CARRIER]** to the sample (and also 2.5 µl Internal Control if using a HUMAN REAL-TIME PCR KITS). Mix by pulse-vortexing 20 times.
- Incubate at 56°C by shaking (500 rpm) for 15 minutes.
- Briefly centrifuge and add 750 µl ethanol (96-100%) **[EtOH]** and mix by pulse-vortexing 20 times and incubate at room temperature (15...25°C) for 3 minutes.
- Transfer 900 µl of the mixture to the column **[COLUMN]** inserted into the collection tube **[COL2ML]**. Centrifuge at 5000 x g for 1 minute. Discard the flow through and insert the column **[COLUMN]** into a new collection tube **[COL2ML]**.
- Repeat step 5 for the remaining mixture. Discard the flow through and insert the column **[COLUMN]** into a new collection tube **[COL2ML]**.
- Add 700 µl Solution W1 **[W-W1]**. Centrifuge at 5000 x g for 1 minute. Discard the flow through and insert the column **[COLUMN]** into a new collection tube **[COL2ML]**.

8. Add 700 µl Solution W2 [W-W2]. Centrifuge at 16000 x g for 1 minute. Discard the flow through and insert the column [COLUMN] into a new collection tube [COL2ML].
9. Repeat step 8 with 700 µl ethanol (96-100 %) [EtOH].
10. Centrifuge at 16000 x g for 3 minutes. Discard the flow through and insert the column [COLUMN] into a new collection tube [COL2ML].
11. Open the tube cap and dry any residual ethanol at 60°C without shaking for 10 minutes.
12. Centrifuge at 16000 x g for 1 minute. Transfer Spin Column [COLUMN] to a 1.5 ml collection tube [COL1.5ML].
13. Add 50 µl of Solution E [E] and incubate at room temperature (15...25°C) for 3 minutes.
14. Centrifuge at 5000 x g for 1 minute, then centrifuge at 16000 x g for additional 30 seconds.
15. Discard the spin column [COLUMN]; the eluate in the microcentrifuge tube contains viral DNA/RNA. Viral DNA/RNA eluate can be used directly for Real-Time PCR applications. Otherwise, store at -20°C or -80°C.

Troubleshooting

Problem	Reason	Solution
Clogged column	Incomplete lysis	Be sure to vortex well at step 3.
Poor or low DNA/RNA recovery	Nuclease contamination	Provide a Nuclease-free working environment. For long-term storage freeze the DNA/RNA eluate at -20°C or -80°C.
	Improper washing	Confirm that the wash solution concentrates [W1] and [W2] were diluted with the volumes of ethanol as indicated. Keep bottles tightly capped between the single working steps to prevent evaporation.
	Poor elution	Repeat the elution step or increase the elution volume.
	Degradation or low concentration of RNA Carrier	Confirm the concentration and storage temperature of the dissolved lyophilized RNA Carrier aliquots. Do not freeze-thaw one aliquot more than 3-4 times.
Downstream applications using the isolated DNA/RNA do not work	DNA/RNA concentration is too low	Precipitate DNA/RNA with ethanol and elute DNA/RNA in a smaller volume of Solution E [E] or dH ₂ O.
	High salt content in the DNA/RNA eluate	Precipitate DNA/RNA with ethanol and elute in Solution E [E] or dH ₂ O.
	Residual ethanol from the diluted wash solution [W-W1] and [W-W2]	Centrifuge the column at 16000 x g for 1 minute after the wash step to remove any residual ethanol.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via:

www.human.de/data/gb/vr/mdx-vnae.pdf or

www.human-de.com/data/gb/vr/mdx-vnae.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Safety Notes

[RL], [W1] Warning!

• Hazard statements

H302 Harmful if swallowed.

H312 Harmful in contact with skin.

H332 Harmful if inhaled.

H413 May cause long lasting harmful effects to aquatic life.

• Precautionary statements

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P322 Specific measures (see on this label).

P301+P312 IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

[PROTEINASE] Danger!

• Hazard statements

H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

• Precautionary statements

P285 In case of inadequate ventilation wear respiratory protection.

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P342+P311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor.

P304+P341 IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

References

1. Knepp J.H. *et al.*, Comparison of Automated and Manual Nucleic Acid Extraction Methods for Detection of Enterovirus RNA, *J. Clin. Microbiol.* **41** (8), 3532-3536 (2003)
2. Casas I. *et al.*, New method for the extraction of viral RNA and DNA from cerebrospinal fluid for use in the polymerase chain reaction assay, *J. Virol. Methods* **53** (1), 25-36 (1995)

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VIRAL DNA ISOLATION KIT

For isolation and purification of viral DNA from human serum or plasma (EDTA) samples for in vitro diagnostic purposes

Package Size

REF	87310	100 Tests	Complete Test Kit
IVD			

Intended Use

VIRAL DNA ISOLATION KIT is an in vitro nucleic acid extraction system to isolate DNA from biological materials. It is intended as sample preparation kit to be used in combination with diagnostic kits such as HUMAN REAL-TIME PCR KITS. Viral DNA is purified from intact cells or cellular structures, which can be further used in sensitive molecular as well as diagnostic downstream applications such as PCR, Real-Time PCR, cloning, RFLP analysis and sequencing. This kit has been validated for use with human serum or human plasma collected in EDTA anticoagulant. VIRAL DNA ISOLATION KIT ([REF](#) 87310) consists of the components TUBES & BUFFERS ([REF](#) 87311) and PROTEINASE K & RNA CARRIER ([REF](#) 87312). These components will be stored differently (see Storage and Stability).

Principle of the test

Isolation of highly-pure viral DNA from serum and plasma samples is essential for clinical diagnosis and molecular research. VIRAL DNA ISOLATION KIT provides a rapid and simple method for the isolation of viral DNA from virus containing cell-free body fluids. As specifically optimized lysis/washing solutions and silica based spin columns designed to capture exclusively viral DNA are employed, high efficiency and purity is achieved by the end of the isolation process.

200 µl serum or plasma sample are used for a single test. Due to the simple procedure of the kit that requires only basic laboratory equipment, it is possible to isolate viral DNA from various samples simultaneously. The recovered viral DNA can be used directly in downstream applications such as RT-PCR, qRT-PCR, qPCR, viral detection, viral load determination and viral genotyping.

The procedure consists of the following steps: Under highly denaturing conditions lysis and inactivation of DNases is accomplished in serum and plasma samples. RNA Carrier is added at start serving to enhance the binding affinity of viral DNAs to the columns and to decrease the degradation possibility of viral DNAs by remaining nucleases. After completion of lysis, the free viral nucleic acid is transferred into columns and the silica gel based membrane captures the viral DNA selectively. In the following consecutive washing steps, contaminants such as proteins, nucleases or inhibitors are efficiently washed away and highly pure viral DNA is eluted.

Reagents and Contents

Components of [REF](#) 87312

CARRIER	1 x vial	RNA Carrier*
PROTEINASE	2 x vials	Proteinase K*

Components of [REF](#) 87311

B	1 x 27.5ml	Solution B
W1	1 x 38ml	Solution W1*
W2	1 x 57ml	Solution W2*
E	1 x 12ml	Solution E
COLUMN	100	Spin Columns
COL2ML	600	Collection tubes (2.0 ml)
COL1.5ML	100	Collection tube (1.5 ml)

*For preparation of RNA Carrier, Proteinase K and the wash buffers [W-W1](#) and [W-W2](#) see "Notes before Starting" section.

Storage and Stability

The components of TUBES & BUFFERS ([REF](#) 87311) of the VIRAL DNA ISOLATION KIT should be stored dry at room temperature (15...25°C). RNA Carrier [CARRIER](#) and Proteinase K [PROTEINASE](#) ([REF](#) 87312) should be stored at -20°C immediately upon arrival. Higher storage temperatures should be avoided. Under these conditions, the kit is stable through the expiration date printed on the label. If solutions of the kit are stored at lower temperatures than suggested, in some of them precipitation may occur, thus causing a decrease in the performance of the kit.

Additional Material Recommended but not Supplied with the Kit:

- 96-100 % ethanol (Molecular Biology grade) [ETOH](#)
- Disposal powder-free gloves
- Micropipettes (0.5 µl-1000 µl) with sterile micropipette tips with filters
- Sterile dH₂O

- Microcentrifuge tubes (2 ml)
- Vortex mixer
- Benchtop microcentrifuge for 2.0 ml tubes and for PCR strip tubes
- Thermomixer

Warnings and Precautions

All clinical specimens and all resulting waste materials should be treated as potentially infectious; the samples should be prepared in Bio-safety Level 2 area.

The procedure should be preferably performed in four separated areas (i.g. for extraction, PCR setup, sample addition and amplification) to avoid contaminations. All items for the particular procedure should be stored in the area where the procedure is performed and should not be exchanged between these areas. The workflow should be unidirectional, beginning in the extraction area followed by downstream application areas.

Before and after work all surfaces should be disinfected with a freshly prepared solution of 10 % bleach or antiviral agents.

Some of the solutions contain guanidinium salts that form reactive compounds and toxic gases if mixed with bleaching or acidic solutions. Do not add bleach or acidic solutions directly to the sample preparation waste.

Handle all materials containing specimens or controls according to Good Laboratory Practice.

Do not mix reagents with different lot numbers or substitute reagents from other manufacturers.

Do not use a kit after its expiration date.

Store the kit away from any source of contaminating DNA, especially amplified DNA.

If the VIRAL DNA ISOLATION KIT substitute another isolation system, both test systems should be used in parallel for at least two subsequent samples.

Notes before Starting

The amount of recovered DNA depends strictly on the nuclease contamination of surroundings. Thus decontamination of hands, plastic and glassware contacting the solutions and working space is very important. Through the procedure, disposable latex or vinyl gloves should be worn all the time, RNase/DNase-free disposable tips and microcentrifuge tubes should be used.

Depending on the ambient temperature precipitation may occur in Solution B [B](#) during long term storage. Before each use, Solution B [B](#) should be checked for precipitates. If precipitation is observed, the solution should be incubated in a 37°C water bath until the solution is homogenous again.

Before use, add 660 µl of Solution E [E](#) to lyophilized RNA Carrier tube [CARRIER](#) and 1100 µl of Solution E [E](#) to one Proteinase K tube [PROTEINASE](#). Mix well by vortexing. The dissolved RNA Carrier solution [W-CARRIER](#) and Proteinase K solutions [W-PROTEINASE](#) should be divided into conveniently sized aliquots and stored at -20°C. The aliquots should not be freeze-thawed more than 3-4 times.

Before use, add 38 ml ethanol (96-100%) [ETOH](#) to Solution W1 [W1](#) and 19 ml ethanol (96-100%) [ETOH](#) to Solution W2 [W2](#) to produce the working solutions [W-W1](#) and [W-W2](#). Solution bottles should be tightly capped between uses to prevent evaporation. The solutions to which ethanol has been added should be kept at room temperature (15...25°C).

Procedure

- Add 20 µl of Proteinase K [W-PROTEINASE](#) and 200 µl of serum or plasma sample into a 1.5 ml tube. If the serum or plasma is frozen, thaw in a 37°C water bath.
- Add 250 µl Solution B [B](#) and 6 µl RNA Carrier [W-CARRIER](#) to the sample (and also 2.5 µl Internal Control if using a HUMAN Real-Time PCR kit). Mix by pulse-vortexing 20 times.
- Incubate at room temperature (15...25°C) by shaking (500 rpm) for 15 minutes.
- Briefly centrifuge and add 200 µl ethanol (96-100 %) [ETOH](#) and mix by pulse-vortexing 20 times and incubate at room temperature (15...25°C) for 3 minutes.
- Transfer the mixture to the column [COLUMN](#) inserted into the collection tube [COL2ML](#). Centrifuge at 5000 x g for 1 minute. Discard the flow through and insert the column [COLUMN](#) into a new collection tube [COL2ML](#).
- Add 700 µl Solution W1 [W-W1](#). Centrifuge at 5000 x g for 1 minute. Discard the flow through and insert the column [COLUMN](#) into a new collection tube [COL2ML](#).
- Add 700 µl Solution W2 [W-W2](#). Centrifuge at 16000 x g for 1 minute. Discard the flow through and insert the column [COLUMN](#) into a new collection tube [COL2ML](#).

8. Repeat step 7 with 700 µl ethanol (96-100 %) [EIOH].
9. Centrifuge at 16000 x g for 3 minutes. Discard the flow through and insert the column [COLUMN] into a new collection tube [COL2ML].
10. Open the tube cap and dry any residual ethanol at 60°C without shaking for 10 minutes.
11. Centrifuge at 16000 x g for 1 minute. Transfer Spin Column [COLUMN] to a 1.5 ml collection tube [COL1.5ML].
12. Add 50 µl of Solution E [E] and incubate at room temperature (15...25°C) for 3 minutes.
13. Centrifuge at 5000 x g for 1 minute, then centrifuge at 16000 x g for additional 30 seconds.
14. Discard the spin column [COLUMN]; the eluate in the microcentrifuge tube contains viral DNA. Viral DNA eluate can be used directly for Real-Time PCR applications. Otherwise, store at -20°C or -80°C.

Troubleshooting

Problem	Reason	Solution
Clogged column	Incomplete lysis	Be sure to vortex well at step 3.
Poor or low DNA recovery	Nuclease contamination	Provide a Nuclease-free working environment. For long-term storage freeze the DNA eluate at -20°C or -80°C.
	Improper washing	Confirm that the wash solution concentrates [W1] and [W2] were diluted with the volumes of ethanol as indicated. Keep bottles tightly capped between the single working steps to prevent evaporation.
	Poor elution	Repeat the elution step or increase the elution volume.
	Degradation or low concentration of RNA Carrier	Confirm the concentration and storage temperature of the dissolved lyophilized RNA Carrier aliquots. Do not freeze-thaw one aliquot more than 3-4 times.
Downstream applications using recovered DNA do not work	DNA concentration is too low	Precipitate DNA with ethanol and elute DNA in a smaller volume of Solution E [E] or dH ₂ O.
	High salt content in the DNA eluate	Precipitate DNA with ethanol and elute in Solution E [E] or dH ₂ O.
	Residual ethanol from the diluted wash solution [W-W1] and [W-W2]	Centrifuge the column at 16000 x g for 1 minute after the wash steps to remove any residual ethanol.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via:

www.human.de/data/gb/vr/mdx-vnae.pdf or

www.human-de.com/data/gb/vr/mdx-vnae.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Safety Notes

[B], [W1] Warning!

• Hazard statements

H302 Harmful if swallowed.

H312 Harmful in contact with skin.

H332 Harmful if inhaled.

H413 May cause long lasting harmful effects to aquatic life.

• Precautionary statements

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P322 Specific measures (see on this label).

P301+P312 IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

[PROTEINASE] Danger!

• Hazard statements

H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

• Precautionary statements

P285 In case of inadequate ventilation wear respiratory protection.

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P342+P311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor.

P304+P341 IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

References

1. Knepp J.H. *et al.*, Comparison of Automated and Manual Nucleic Acid Extraction Methods for Detection of Enterovirus RNA, J. Clin. Microbiol. **41** (8), 3532-3536 (2003)
2. Casas I. *et al.*, New method for the extraction of viral RNA and DNA from cerebrospinal fluid for use in the polymerase chain reaction assay, J. Virol. Methods **53** (1), 25-36 (1995)

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VIRAL RNA ISOLATION KIT

For isolation and purification of viral RNA from human serum or plasma (EDTA) samples for in vitro diagnostic purposes

Package Size

[REF]	87320	100 Tests	Complete Test Kit
[IVD]			

Intended Use

VIRAL RNA ISOLATION KIT is an in vitro nucleic acid extraction system to isolate RNA from biological materials. It is intended as sample preparation kit to be used in combination with diagnostic kits such as the HUMAN REAL-TIME PCR KITS. Viral RNA is purified from intact cells or cellular structures, which can be further used in sensitive molecular as well as diagnostic downstream applications such as PCR, Real-Time PCR, cloning, RFLP analysis and sequencing. This kit has been validated for use with human serum or human plasma collected in EDTA anticoagulant. VIRAL RNA ISOLATION KIT ([REF] 87320) consists of the components TUBES & BUFFERS ([REF] 87321) and RNA CARRIER ([REF] 87322). These components will be stored differently (see "Storage and Stability").

Principle of the test

Isolation of highly-pure viral RNA from serum and plasma samples is essential for clinical diagnosis and molecular research. VIRAL RNA ISOLATION KIT provides a rapid and simple method for the isolation of viral RNA from virus containing cell-free body fluids. As specifically optimized lysis/washing solutions and silica based spin columns designed to capture exclusively viral RNA are employed, high efficiency and purity is achieved by the end of the isolation process.

150 µl serum or plasma sample are used for a single test. Due to the simple procedure of the kit that requires only basic laboratory equipment, it is possible to isolate viral RNA from various samples simultaneously. The recovered viral RNA can be used directly in downstream applications such as RT-PCR, qRT-PCR, qPCR, viral detection, viral load determination and viral genotyping.

The procedure consists of the following steps: Under highly denaturing conditions lysis and inactivation of nucleases is accomplished in serum and plasma samples. RNA Carrier is added at start serving to enhance the binding affinity of viral RNAs to the columns and to decrease the degradation possibility of viral RNAs by remaining nucleases. After completion of lysis, the free viral RNA is transferred into columns and the silica gel based membrane captures the viral RNAs selectively. In the following consecutive washing steps, contaminants such as proteins, nucleases or inhibitors are efficiently washed away and highly pure viral RNA is eluted.

Reagents and Contents

Components of [REF] 87322

[CARRIER]	1 x vial	RNA Carrier*
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Components of [REF] 87321

[RL]	1 x 66ml	Solution RL
[W1]	1 x 38ml	Solution W1*
[W2]	1 x 15ml	Solution W2*
[E]	1 x 12ml	Solution E
[COLUMN]	100	Spin Columns
[COL2ML]	700	Collection tubes (2.0 ml)
[COL1.5ML]	100	Collection tube (1.5 ml)

*For preparation of RNA Carrier and the wash solutions [W-W1] and [W-W2] see "Notes before Starting" section.

Storage and Stability

The components of TUBES & BUFFERS ([REF] 87321) of the VIRAL RNA ISOLATION KIT should be stored dry at room temperature (15...25°C). RNA Carrier [CARRIER] ([REF] 87322) should be stored at -20°C immediately upon arrival. Higher storage temperatures should be avoided. Under these conditions, the kit is stable through the expiration date printed on the label. If solutions of the kit are stored at lower temperatures than suggested, in some of them precipitation may occur, thus causing a decrease in the performance of the kit.

Additional material recommended but not supplied with the kit:

- 96-100 % ethanol (Molecular Biology grade) [EtOH]
- Disposal powder-free gloves
- Micropipettes (0.5 µl-1000 µl) with sterile micropipette tips with filters
- Sterile dH₂O
- Microcentrifuge tubes (2.0 ml)

- Vortex mixer
- Benchtop microcentrifuge for 2.0 ml tubes
- Thermomixer

Warnings and Precautions

All clinical specimens and all resulting waste materials should be treated as potentially infectious; the samples should be prepared in Bio-safety Level 2 area.

The procedure should be preferably performed in four separated areas (i.g. for extraction, PCR setup, sample addition and amplification) to avoid contaminations. All items for the particular procedure should be stored in the area where the procedure is performed and should not be exchanged between these areas. The workflow should be unidirectional, beginning in the extraction area followed by downstream application areas.

Before and after work all surfaces should be disinfected with a freshly prepared solution of 10 % bleach or antiviral agents.

Some of the solutions contain guanidinium salts that form reactive compounds and toxic gases if mixed with bleaching or acidic solutions. Do not add bleach or acidic solutions directly to the sample preparation waste.

Handle all materials containing specimens or controls according to Good Laboratory Practice (GLP).

Do not mix reagents with different lot numbers or substitute reagents from other manufacturers.

Do not use a kit after its expiration date.

Store the kit away from any source of contaminating DNA or RNA, especially amplified nucleic acids.

If the VIRAL RNA ISOLATION KIT substitutes another isolation system, both test systems should be used in parallel for at least two subsequent samples.

Notes before Starting

The amount of recovered RNA depends strictly on the nuclease contamination of surroundings. Nucleases are very stable enzymes and just need minutes to degrade RNAs in short pieces. Thus, decontamination of hands, plastic and glassware contacting the solutions and working space is very important. Through the procedure, disposable latex or vinyl gloves should be worn all the time, RNase/DNase-free disposable tips and microcentrifuge tubes should be used.

Depending on the ambient temperature precipitation may occur in Solution RL [RL] during long term storage. Before each use, Solution RL [RL] should be checked for precipitates. If precipitation is observed, the solution should be incubated in a 37°C water bath until solution is homogenous again.

Before use, add 660 µl of Solution E [E] to lyophilized RNA Carrier tube [CARRIER]. Mix well by vortexing. The dissolved RNA Carrier solution [W-CARRIER] should be divided into conveniently sized aliquots and stored at -20°C. The aliquots should not be freeze-thawed more than 3-4 times.

Before use, add 38 ml ethanol (96-100 %) [EtOH] to Solution W1 [W1] and 60 ml ethanol (96-100 %) [EtOH] to Solution W2 [W2] to produce the working solutions [W-W1] and [W-W2]. Solution bottles should be tightly capped between uses to prevent evaporation. The solutions to which ethanol has been added should be kept at room temperature (15...25°C).

Procedure

- Add 150 µl of serum or plasma sample into a 1.5 ml tube. If the serum or plasma is frozen, thaw in a 37°C water bath.
- Add 600 µl Solution RL [RL] and 6 µl RNA Carrier [W-CARRIER] to the sample (and also 2.5 µl Internal Control if using a HUMAN REAL-TIME PCR KITS). Mix by pulse-vortexing 20 times.
- Incubate at room temperature (15...25°C) by shaking (500 rpm) for 15 minutes.
- Briefly centrifuge and add 600 µl ethanol (96-100 %) [EtOH] and mix by pulse-vortexing 20 times and incubate at room temperature (15...25°C) for 3 minutes.
- Transfer 700 µl of the mixture to the column [COLUMN] inserted into the collection tube [COL2ML]. Centrifuge at 5000 x g for 1 minute. Discard the flow through and insert the column [COLUMN] into a new collection tube [COL2ML].
- Repeat step 5 for the remaining mixture. Discard the flow through and insert the column [COLUMN] into a new collection tube [COL2ML].
- Add 700 µl Solution W1 [W-W1]. Centrifuge at 5000 x g for 1 minute. Discard the flow through and insert the column [COLUMN] into a new collection tube [COL2ML].
- Add 700 µl Solution W2 [W-W2]. Centrifuge at 16000 x g for 1 minute. Discard the flow through and insert the column [COLUMN] into a new collection tube [COL2ML].
- Repeat step 8 with 700 µl ethanol (96-100 %) [EtOH].

10. Centrifuge at 16000 x g for 3 minutes. Discard the flow through and insert the column [COLUMN] into a new collection tube [COL2ML].
11. Open the tube cap and dry any residual ethanol at 60°C without shaking for 10 minutes.
12. Centrifuge at 16000 x g for 1 minute. Transfer Spin Column [COLUMN] to a 1.5 ml collection tube [COL1.5ML].
13. Add 50 µl of Solution E [E] and incubate at room temperature (15...25°C) for 3 minutes.
14. Centrifuge at 5000 x g for 1 minute, then centrifuge at 16000 x g for additional 30 seconds.
15. Discard the spin column [COLUMN]; the eluate in the microcentrifuge tube contains viral RNA. Viral RNA eluate can be used directly for Real-Time PCR applications. Otherwise, store at -20°C or -80°C.

Troubleshooting

Problem	Reason	Solution
Clogged column	Incomplete lysis	Be sure to vortex well at step 3.
Poor or low RNA recovery	Nuclease contamination	Provide a Nuclease-free working environment. For long-term storage freeze the RNA elute at -20°C or -80°C.
	Improper washing	Confirm that the wash solution concentrates [W1] and [W2] were diluted with the volumes of ethanol as indicated. Keep bottles tightly capped between the single working steps to prevent evaporation.
	Poor elution	Repeat the elution step or increase the elution volume.
	Degradation or low concentration of RNA Carrier	Confirm the concentration and storage temperature of the dissolved lyophilized RNA Carrier aliquots. Do not freeze-thaw one aliquot more than 3-4 times.
Downstream applications using the isolated RNA do not work	RNA concentration is too low	Precipitate RNA with ethanol and elute RNA in a smaller volume of Solution E [E] or dH ₂ O.
	High salt content in the DNA/RNA eluate	Precipitate RNA with ethanol and elute in Solution E [E] or dH ₂ O.
	Residual ethanol from the diluted wash solution [W-W1] and [W-W2]	Centrifuge the column at 16000 x g for 1 minute after the wash step to remove any residual ethanol.

Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via:

www.human.de/data/gb/vr/mdx-vnae.pdf or

www.human-de.com/data/gb/vr/mdx-vnae.pdf

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

Safety Notes

[RL], [W1] Warning

• Hazard statements

H302 Harmful if swallowed.

H312 Harmful in contact with skin.

H332 Harmful if inhaled.

H413 May cause long lasting harmful effects to aquatic life.

• Precautionary statements

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P322 Specific measures (see on this label).

P301+P312 IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

References

1. Knepp J.H. *et al.*, Comparison of Automated and Manual Nucleic Acid Extraction Methods for Detection of Enterovirus RNA, J. Clin. Microbiol. **41** (8), 3532-3536 (2003)
2. Casas I. *et al.*, New method for the extraction of viral RNA and DNA from cerebrospinal fluid for use in the polymerase chain reaction assay, J. Virol. Methods **53** (1), 25-36 (1995)

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