Instructions for Use

Ver. 1 - 18/03/2020

Molecular biology

Viral DNA/RNA extraction

Magnetic beads

REF AA1318

Σ 48 TESTS

EDMA 1590400

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ISO 9001 Quality Management System Organization certified and ISO 13485 Medical Sector Quality Management System certified (IMQ certification body - CSQ certification).

INTENDED USE

⇨ This device allows the isolation of highly pure viral DNA/RNA from human serum, plasma or swab. The obtained DNA/RNA is ready to use for downstream reactions and analysis.

PRINCIPLE OF THE PROCEDURE

⇨ Viral DNA/RNA can be isolated from human serum, plasma or swab. Lysis is achieved by incubation of samples in a solution containing Proteinase K and Poly(A) RNA at +55°C. Appropriate DNA/RNA binding conditions to the Magnetic Beads Viral DNA/RNA are created by addition of Binding Buffer Viral DNA/RNA to the lysate; the binding process is reversible and specific for nucleic acids. Washing steps efficiently remove contaminants. Pure viral DNA/RNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

KIT CONTENTS

Reagents				NLM code	Quantity
Lysis Buffer Viral RNA	Guanidine thiocyanate 60-80% CAS: 593-84-0	 WARNING	**H302, H312, H332** P261, P264, P270, P271, P280, P301+P312, P302+P352, P304+P340, P312, P322, P330, P363, P501	MA1213	27 ml
Lysis Buffer Viral DNA	Guanidinium chloride 40-60% CAS: 50-01-1	 WARNING	**H302, H315, H319** P264, P270, P280, P301+P312, P302+P352, P305+P351+P338, P330, P332+P313, P337+P313, P362, P501	MA1283	27 ml
Binding Buffer Viral DNA/RNA	Sodium perchlorate 30-50% CAS: 7601-89-0 Ethanol 50-70% CAS: 64-17-5	 WARNING DANGER	**H225, H302** P210, P233, P240, P241, P242, P243, P264, P270, P280, P301+P312, P303+P361+P353, P330, P370+P378, P403+P235, P501	MA1214	76 ml
Wash Buffer A Viral DNA/RNA	Sodium perchlorate < 25% CAS: 7601-89-0 Ethanol 30-50% CAS: 64-17-5	 WARNING	**H226** P210, P233, P240, P241, P242, P243, P280, P303+P361+P353, P370+P378, P403+P235, P501	MA1215	30 ml
Wash Buffer B Viral DNA/RNA	Ethanol 60-80% CAS: 64-17-5	 DANGER	**H225** P210, P233, P240, P241, P242, P243, P280, P303+P361+P353, P370+P378, P403+P235, P501	MA1216	30 ml
Elution Buffer Viral DNA/RNA			-	MA1217	4x1,55 ml
Magnetic Beads Viral DNA/RNA			-	MA1218	4x0,5 ml
Water (RNase, DNase, Protease free)			-	AZ040	2x1,5 ml
Poly(A) RNA Buffer	Guanidine thiocyanate 30-50% CAS: 593-84-0	 WARNING	**H302** P264, P270, P301+P312, P330, P501	MA1220	0,5 ml
Lyophilized Proteinase K	Proteinase K Tritirachium album serina 90-100% CAS: 39450-01-6	 WARNING DANGER	**H315, H319, H334, H335,** P261, P264, P271, P280, P285, P302+P352, P304+P340, P304+P341, P305+P351+P338, P312, P332+P313 P337+P313 P342+P311, P362, P403+P233, P405, P501	MA1221	1 vial
Lyophilized Poly(A) RNA			-	MA1219	1 vial

PREPARATION AND STORAGE OF WORKING SOLUTIONS

Before using the kit for the first time prepare the reagents as follows:

Proteinase K: add 2,5 ml of molecular grade water (DNase, RNase and Protease free, supplied) to dissolve lyophilized Proteinase K. Proteinase solution is stable at +2/+8°C for 2 weeks. Storage of adequate aliquots at -25/-15°C is recommended if the solution will not be used up during this period. Do not re-freeze the Proteinase K aliquots after thawing.

Poly(A) RNA: add 440 µl of Poly(A) RNA Buffer to each tube of Lyophilized Poly(A) RNA. This solution is stable at +2/+8°C for 4 weeks. Storage of adequate aliquots at -25/-15°C is recommended if the solution will not be used up during this period. Do not re-freeze the Poly(A) RNA aliquots after thawing.

Lysis Buffers, **Wash Buffers Viral DNA/RNA, Binding Buffer Viral DNA/RNA, Elution Buffer Viral DNA/RNA** and the **Magnetics Beads Viral DNA/RNA** must be stored at room temperature.

Store the **Lysis Buffer Viral RNA, Lysis Buffer Viral DNA** and the **Poly(A) RNA Buffer** in the dark. **Lysis Buffer Viral RNA** and **Lysis Buffer Viral DNA** may form a precipitate upon storage. If necessary, warm to +55°C to dissolve.

Binding Buffer Viral DNA/RNA, Wash Buffer A Viral DNA/RNA and **Wash Buffer B Viral DNA/RNA** contain ethanol. Longer storage of the buffers without lids should be avoided. If ethanol evaporates the optimal yield cannot be guaranteed.

The **Magnetic Beads Viral DNA/RNA** suspension should be mixed vigorously before dispensing, otherwise the suspension is not homogenous and the NA yield could be low.

The **Elution Buffer Viral DNA/RNA** included in this kit is 10 mM Tris-HCl pH 8.0. TE buffer pH 8.0 can also be used without any protocol adjustments.

UV MEASUREMENTS

In some cases it is possible to find traces of **Magnetic Beads Viral DNA/RNA** left in the eluate. Such particles will not interfere with PCR and most downstream applications but may increase the background UV measurements. In such a case, prior to UV analysis, it is recommend an additional separation step using a manual separator in order to separate any traces of particles.

⇨ STORAGE OF HUMAN SAMPLES

Serum/plasma
Store samples at +2/+8°C until extraction step. If they are not processed immediately store them in tubes at -25/-15°C for up to 72 hours prior to freezing at -80°C. In this case, thaw the sample at +2/+8°C slowly before use.

Swabs
Swabs must be prepared ad stored according to laboratory specifications

PRECAUTIONS

- Only professional and opportunely trained personnel should use this kit. Handle this product according to established good laboratory practices and universal precautions throughout the assay procedure.
- The use of the following solutions allows to minimize the risk of cross-contamination:
  - ✓ It is recommended to perform the assay in three different areas:
    - Area 1: pre-PCR (samples handling and extraction)
    - Area 2: Master Mix preparation and sample addition to the RT-PCR mix.
    - Area 3: post-PCR (Real Time PCR)

    Each area should be provided with dedicated equipment and consumables (lab coats, centrifuge, tubes, pipettes, etc).

    Workflow must proceed in a unidirectional manner, beginning in the RNA extraction area (Area 1), moving to the mastermix preparation and RNA addition area (area 2) and finally to amplification and detection area (Area 3). This means that consumables and PPE (lab coats, gloves, etc.) that have been introduced into the post-PCR room should never be placed back to the pre-PCR room without thorough decontamination.
  - ✓ It's recommended to clean the workstation with 5-10% bleach (final concentration of sodium hypoclorite: 0,5% w/v) at the end of the procedure. Prepare bleach solution daily.
  - ✓ All disposable items (tips and tubes) must be DNase, RNase free. Use aerosol-resistant pipette tips to avoid pipettes contamination. Use a new tip every time a volume is dispensed.
  - ✓ Work using vertical downflow airbox with UV lamp
  - ✓ Change gloves frequently
  - ✓ The absence of contamination is guaranteed by the analysis in each session of a negative control, which allows to monitor the whole procedure starting from the extraction step
- Discard all used material in accordance with the existing local and national regulations in force
- Do not eat, drink, smoke or apply cosmetics in areas where reagents or specimens are handled.
- Do not use device after its expiration date.
- Do not mix reagents from different lots.
- Do not use the device if the box is damaged; contact the supplier
- Only for automatic extraction: pay attention to device modularity: if the modularity is not respected the reagents could be insufficient for all the tests.
- **It is advisable to have constant and uniform laboratory temperature, avoid to place the instruments near heating/cooling sources that may compromise the right working.**

- **Hazard statements**:
  - **H225:** Highly flammable liquid and vapour.
  - **H226:** Flammable liquid and vapour.
  - **H302:** Harmful if swallowed.
  - **H312:** Harmful in contact with skin.
  - **H315:** Causes skin irritation.
  - **H319:** Causes serious eyes irritation.
  - **H334:** May cause allergy or asthma symptoms or breathing difficulties if inhaled.
  - **H335:** May cause respiratory irritation.
- **Precautionary statements**:
  - **P210:** Keep away from heat/sparks/open flames/hot surfaces. — No smoking.
  - **P233:** Keep container tightly closed.
  - **P240:** Ground/bond container and receiving equipment.
  - **P241:** Use explosion-proof electrical/ventilating/lighting/…/equipment.
  - **P242:** Use only non-sparking tools.
  - **P243:** Take precautionary measures against static discharge.
  - **P261:** Avoid breathing dust/fume/gas/mist/vapours/spray.
  - **P264:** Wash … thoroughly after handling.
  - **P270:** Do no eat, drink or smoke when using this product.
  - **P271:** Use only outdoors or in a well-ventilated area.
  - **P280:** Wear protective gloves/protective clothing/eye protection/face protection.
  - **P285:** In case of inadequate ventilation wear respiratory protection.
  - **P301+P312:** IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
  - **P302+P352:** IF ON SKIN: Wash with plenty of soap and water.
  - **P303+P361+P353:** IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
  - **P304+P340:** IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
  - **P304+P341:** IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing.
  - **P305+P351+P338:** IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing
  - **P312:** Call a POISON CENTER or doctor/physician if you feel unwell.
  - **P322:** Specific measures (see MSDS).
  - **P330:** Rinse mouth.
  - **P337+P313:** If eye irritation persists: Get medical advice/attention.
  - **P342+P311:** If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.
  - **P362:** Take off contaminated clothing and wash before reuse.
  - **P363:** Wash contaminated clothing before reuse.
  - **P370+P378:** In case of fire: Use … for extinction. (see MSDS).
  - **P403+P233:** Store in a well-ventilated place. Keep container tightly closed.
  - **P403+P235:** Store in a well-ventilated place. Keep cool.
  - **P405:** Store locked up.
  - **P501:** Dispose of contents/containers as biohazardous waste.

MATERIAL REQUIRED BUT NOT PROVIDED

Vertical downflow airbox
Precision pipettes set
DNase and RNase free aerosol barrier tips and 2 ml micro-centrifuge tubes
DNase and RNase free tubes for storage
Microcentrifuge
Vortex mixer and Heat block
Magnetic separator (e.g. chemagic Stand 2x12 Perkin Elmer)
or
Janus Automated Workstation

VIRAL DNA/RNA PURIFICATION PROTOCOL

WARNING

- Use **Lysis Buffer Viral RNA** in case of viral RNA extraction
- Use **Lysis Buffer Viral DNA** in case of viral DNA extraction

⇨ • In case of isolation from swabs, make sure that the transport medium used is compatible with molecular biology and does not contain PCR inhibitors that may influence downstream applications.

MANUAL EXTRACTION

Before starting with the procedure set heat block to +55°C.
Ensure that Proteinase K and Poly(A) RNA have been prepared as described.
Prepare the necessary amount of 2 ml centrifuge tubes (samples and negative control).

1. Dispense **in the following order 10 µl Proteinase K** and **450 µl** of **sample** into 2,0 ml centrifuge tubes. Add **450 µl Lysis Buffer Viral** and then **6 µl Poly(A) RNA**; proceed pipetting up and down for at least **8 times** and incubate at **+55°C** for **10 minutes**, vortexing once or twice during incubation.
   It is also possible to prepare a premix of Lysis Buffer Viral and Poly(A) RNA, calculating the necessary amount for the number of samples to process +1. Use this mix within 15 minutes.
2. Following incubation, briefly centrifuge the sample to avoid contamination when opening the tubes. Add **35 µl** of resuspended **Magnetic Beads Viral DNA/RNA (the Magnetic Bead suspension should be mixed vigorously before dispensing)** and **900 µl of Binding Buffer Viral DNA/RNA** to each tube, mix by pipetting up and down for at least **6 times** and incubate for **5 minutes** at RT.
   It's possible to prepare a premix of beads and Binding Buffer Viral DNA/RNA.
3. Place the tube in a magnetic separator for **2 minutes** to let the beads be attracted by the magnets. Remove and discard supernatant by pipetting, **being careful not to take any beads.**
4. Remove the tubes from the magnetic separator. Add **500 µl Wash Buffer A Viral DNA/RNA** to the tube. Resuspend the beads by pipetting until the beads are completely resuspended and incubate for **1 minute** at RT.
5. Place the tube in the magnetic separator to draw the beads to the side of the tube (about 1 minute). Pipette off the supernatant and then remove the tube from the magnet.
6. Repeat the washing procedure (steps 4 and 5) with **500 µl Wash Buffer B Viral DNA/RNA.**
   Remove all traces of Wash Buffer B Viral DNA/RNA repeating the aspiration with a fine-tipped pipette; be careful to remove all the residual fluid without disturbing the pellet.
7. Transfer the tubes directly from the magnetic position to heat block and let air-dry each tube at **+55°C** for **20 minutes** to remove any traces of ethanol. The beads-pellet should become light brown when dried; otherwise extend the incubation for a further 5 minutes or until completely dry.
   It is important that the beads are completely dried and that there aren't ethanol drops on the tube walls before continuing with the elution step, as ethanol can prevent amplification

8. Add **70 µl** of **Elution Buffer Viral DNA/RNA** to the tubes and resuspend the beads pellet by pipetting, avoiding the residues of salts present on the walls of the tube. Incubate the suspension for **5 minutes** at **+55°C**, to facilitate complete DNA/RNA elution.
9. Separate the magnetic beads by placing the tubes in the magnetic separator for at least **1 minute**. Transfer the supernatant containing the purified viral DNA/RNA to the desired storage tubes.

Purified DNA/RNA can be stored at +2/+8°C if immediately used; otherwise keep it frozen at -25/-15°C or at ≤-70°C for longer periods. It is recommended to thaw at +2/+8°C.

AUTOMATIC EXTRACTION WITH JANUS (Perkin Elmer)

MODULARITY

Reagents are provided for 3 distinct runs. If modularity isn't respected, reagents could be not enough for the number of tests declared.

PROCEDURES

Program to be used: ***Viral DNA/RNA***

Important:

- Switch on the heater plate **(+75°C)** 20 minutes before starting the protocol
- Mix vigorously the tubes containing the beads before starting the extraction.

Required material for AA1318 extraction

- 2 deepwell plate 48 wells, 5 ml (for each extraction run)
- Strips or plate to collect the extracts
- 5 trays for the extraction liquids
- 1,5 ml tubes for Proteinase K and Poly(A)RNA aliquots (use 2 aliquots of Proteinase K and 2 of Poly(A)RNA for each extraction run)
- Tips and Magnetic Beads tubes:

Samples	n° Magnetic Beads Viral DNA/RNA tubes	n° TIP 900µl	n° TIP 175µl	n° TIP 25µl
1	1	7	3	3
2	2	14	6	6
3	3	21	9	9
4	4	28	12	12
8	4	52	20	20
12	4	64	28	20
24	4	124	52	32
48	4	244	100	56

For instruction for use, maintenance and precautions please refer to the Instrument manual.

WARNING: Circular shape colored labels to be applied to the instrument trays for visually identification of reagents are provided in the kit. The same colors are visible on the liquid packaging labels in the kit, according to the color code shown in the reagent name list below. Four labels for each color are provided inside kit.

1_ Lysis Buffer Viral RNA (Only in case of viral RNA extraction)

2_ Lysis Buffer Viral DNA (Only in case of viral DNA extraction)

3_ Binding Buffer Viral DNA/RNA

4_ Wash Buffer A Viral DNA/RNA

5_ Wash Buffer B Viral DNA/RNA

TROUBLESHOOTING

Problem	Possible cause and suggestions
No/poor DNA/RNA yield or quality	<u>Incomplete cell lysis</u> - Sample not thoroughly mixed with lysis buffer/Proteinase K and Poly(A) RNA. The mixture has to be mixed immediately after addition of the sample. - Proteinase K digestion not optimal. Be sure that the heat block has been set to +55°C (75°C for Janus). <u>Reagents not applied properly</u> - Prepare Proteinase K solution and Poly(A) RNA according to instructions. - The Magnetic Beads suspension should be mixed vigorously before dispensing, otherwise the suspension is not homogenous and the NA yield could be low. <u>Suboptimal elution of DNA/RNA from the beads</u> - Be sure to remove all the ethanol-containing buffers before eluting the DNA/RNA. If the pellet of beads has not become light brown, incubate at +55°C again until the pellet has completely dried. - Apply elution buffer directly on the pellet of beads and resuspending by pipetting.