Peps6-CaptoVIR kit

Reference: MP10042

For research use only

	Expiration date
2-8°C	Store at temperature range 2°C to 8°C
LOT	Lot number
REF	Reference number

technologies ApoH

Increasing sensitivity, improving diagnostics

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1 – INTRODUCTION

The **synthetic** molecule called Peps6 is derived from the Apolipoprotein H protein (ApoH) and retains its ability to bind micro-organisms including **viruses** (1-2), **fungi** (3) and **bacteria** (4-6). The ApoH protein is also known as Apolipoprotein H or Beta-2 glycoprotein 1. Its poly-specific nature allows **multiplexing** of various micro-organisms. This affinity capture method proves to be **simple, soft and fast** enough so that the micro-organisms retain their viability and infectivity. The captured micro-organisms are concentrated and **separated from potential inhibitors** and so become easier to identify/detect by the usual specific techniques, leading to a gain of sensitivity (7-10).

These characteristics make the Peps6-CaptoVIR kit an **innovative** sample pretreatment tool for the isolation of viruses before their **sensitive** identification/detection.

2 – PRINCIPLE

In the Peps6-CaptoVIR kit, Peps6 is bound to magnetic beads. The kit is supplied with special binding buffers that increase the affinity of the Peps6 towards viruses. The Peps6 magnetic beads are added to any complex biological medium, diluted in the supplied buffer. The initial sample and its potential inhibitors can be removed whereas the captured viruses, linked via Peps6 to the magnetic beads, are kept in the test tube using a magnet. Viruses are then ready to be processed for their identification-detection by methods such as molecular techniques (PCR), immunological detection (ELISA, WB) or culture in appropriate cells.

3 – REAGENTS

REF MP20006 – Peps6 magnetic beads

The suspension of synthetic Peps6-coated magnetic particles corresponds to 10^{13} beads/mL of ~200 nm diameter beads in a buffer containing < 0.02% sodium azide.

REF TP10002 – Buffer TAS 20X

The Buffer TAS is a clear binding buffer concentrated 20X. Dilute according to the instructions below.

REF TP10007 – Additive FS 100X

Additive FS is supplied as a light-sensitive powder. Dilute with Solvent FS 100X according to the instructions below.

REF TP10008 – Solvent FS 100X

Solvent FS is an aqueous solution for the resuspension of Additive FS concentrated 100X. Do not dilute before adding to Additive FS.

Note: all reagents included in the kit are available separately.

4 – STORAGE

- All reagents may travel at ambient temperature without altering their function; store at 2-8°C upon reception.
- All unopened reagents remain stable at 2-8°C until the expiration date. When opened, all reagents should be rapidly stored at 2-8°C, except the liquid form of Additive FS which must be stored at -20°C.
- The Additive FS, in **solid form**, is stable at 2-8°C until the expiration date. After resuspension in Solvent FS, it is light-sensitive and heat-sensitive and must be stored in its original amber tube tightly closed at -20°C. When frozen, the **liquid form** of Additive FS is stable at -20°C until the expiration date.
- The combination of Buffer TAS, Additive FS and Solvent FS (named Binding Buffer) should be used immediately; do not store for future use.
- The Peps6 magnetic beads vial should be stored upright to always keep beads within their storage solution.

5 – MATERIAL REQUIRED, NOT PROVIDED

- Sterile osmosed water.
- Suitable micropipettes and filter tips.
- Suitable reaction tubes, glass or plastic (polypropylene only, avoid polystyrene).
- Suitable equipment for the sample agitation during incubation.
- Incubator regulated at the appropriate temperature.
- Laminar flow hood or any particular microbiologic environment required by the type of micro-organism targeted.
- Magnetic device dedicated to lateral attraction compatible with the test tube; please note that **attraction efficiency and speed vary between different commercially-available magnets**.
- Materials and reagents required for the revelation of targeted micro-organisms.

6 – SAFETY AND PRECAUTIONS

- For better stability, all reagents must be handled with care to **avoid any contaminations**.
- The need for a **sterile work area** will be determined by the use of captured micro-organisms (mandatory for culture).

- The Peps6 magnetic beads storage buffer contains < 0.02% sodium azide. Traces of sodium azide do not interfere with capture, nor with micro-organism viability: there is no need to wash the beads prior to use. Sodium azide may react with copper or lead plumbing to form explosive metal azides. When disposing through plumbing, flush with large volumes of water to prevent azide accumulation.

- Reagents and specimens should be handled in accordance to good laboratory practices. Dispose of unused reagents, samples and wastes in accordance with local regulations.
- Do not use out-of-date reagents.

7 – IMPORTANT NOTES

This protocol is intended to provide general guidelines for the binding of viruses in up to 2 mL samples. Some viruses do not require the Additive FS, recommended in the first trials. Further optimization may be required in order to achieve optimal binding capacity depending on the virus type, sample nature and volume.
The mechanism of ApoH/Peps6 capture **differs** from regular antibody-antigen interactions. To ensure **better success** in your trials, contact our technical support:
info@apohtech.com

- The Peps6 magnetic beads **must not be vortexed**, frozen, dried, handled at high temperatures (> 60°C) or extreme pH (>9 or <5), prior to viral capture. Same care should be taken after capture if retaining infectious viruses is an issue.
- Increase bead volume only if high viral loads are suspected. Beads are able to bind high numbers of viruses: 10 µL of beads bind over 1E+8 Ebola viruses in a clinical human plasma sample.

8 – SAMPLE COLLECTION AND HANDLING

Our current data show that the Peps6 magnetic beads can capture viruses in all kinds of solid (after suspension) or liquid samples.
- Grind your solid sample (ex: meat, tissue) in TAS buffer diluted to 1X. Spin or filter the sample on a sterile gauze. Harvest supernatant or filtrate, to remove sample pieces that will interfere with bead magnetization.
- Use preferentially fresh samples and avoid pooling them. Pooled blood, pooled serum or pooled plasma may create a coagulum that is able to trap and aggregate the magnetic beads, which are then not available to bind micro-organisms.
- When using or plasma, choose the **EDTA anti-coagulant**.
- All diluted or treated samples should be rapidly put in contact with the Peps6 magnetic beads.
- Sample volume may be scaled up or down. Scale up sample volume if low viral titers are suspected. For volumes above 2 mL, contact our technical support.

Damaged viruses may lose their affinity to the ApoH protein or the ApoH-derived Peps6 molecule, so:
- Use preferably fresh material or samples that have been immediately frozen and stored at -20°C or -80°C. **Repeated freeze-thaw cycles** of samples should be avoided.
- Never use **inactivated viruses**.
- Use of poor-quality starting material leads to reduced sensitivity.

9 – INSTRUCTIONS FOR USE

Preparing the liquid form of Additive FS concentrated 100X
Add 0.5 mL Solvent FS 100X into the tube of Additive FS 100X and vortex for 1 full minute, both upright and upside down. Leave the tube at room temperature for 10 minutes and vortex again for 1 full minute.

Sample dilution in binding buffer
- Dilute Buffer TAS 20X to 1X concentration in sterile osmosed water. Vortex.
- Dilute sample 5 fold in binding buffer: add 1 volume of sample + 4 volumes of Buffer TAS 1X + (1/20th) 0.05 volume of liquid Additive FS 100X.
- Vortex sample after dilution.

Capture
- Prior to use, thoroughly resuspend the Peps6 magnetic beads by gentle pipetting or manual inversion of the vial (do not vortex).
- Add 10 µL Peps6 magnetic beads per sample.
- Gently homogenize (do not vortex).
- Incubate for 30 min at 2-8°C under proper agitation: tubes should be kept up-right with vigorous agitation so the Peps6 magnetic beads remain in suspension. For example, set a Thermomixer to 1000 rpm.
- Place the reaction tube on a magnet until all Peps6 magnetic beads are laterally pelleted and the supernatant has cleared up.
- Discard supernatant without disturbing the pellet of Peps6 magnetic beads. **Viruses are now concentrated in the pellet.**

Washing (optional)
Washing is not needed for cell supernatant, blood plasma or serum. Complex samples like whole blood may require washes:
- Gently add 1 mL of Buffer TAS diluted 1X on the beads pelleted on the magnet. Don't suspend the beads.
- Discard supernatant without disturbing the pellet of Peps6 magnetic beads.
- Repeat washing procedure once if needed.

10 – VIRAL DETECTION

The bound viruses can be revealed directly on the Peps6 magnetic beads using your standard protocols, which may be adapted if necessary. Note: For low volumes, short spin the bead pellet before adding resuspension solution.

PCR: Resuspend the Peps6 magnetic beads in your lysis buffer. Vortex vigorously during 15 seconds to disrupt bead pellet. After the lysis step, and before adding ethanol or chloroform, remove the Peps6 magnetic beads on a magnet or by centrifugation 1 min at 10 000 g. Transfer the supernatant in a new tube. Proceed with your usual DNA/RNA extraction and (RT)-PCR protocol.

Microscopy: Resuspend the Peps6 magnetic beads in PBS or your specific media. Beads are not auto-fluorescent and can be used for fluorescent applications.

Culture: Resuspend the Peps6 magnetic beads in the appropriate media. Add directly the suspension in a compatible cell culture.

Other: Resuspend the Peps6 magnetic beads in an appropriate solution for other applications. Please contact our technical support for other specific applications.

11 – TROUBLESHOOTING

Some guidelines are given below. Please contact our technical support for any remaining questions, for further information or for protocols tailored to your specific application:

info@apohtech.com

Samples

- To optimize the availability of certain micro-organisms in solid samples, grinding may be performed in CTAB instead of TAS Buffer [CTAB: 3 % cetyltrimethyl ammonium bromide, 1M Tris base, 1,4 M NaCl, 20 mM EDTA]. Contact our technical support to check the compatibility of other sample lysis buffers.

Large sample volumes

- Large sample volumes (2-100 mL) require more beads and buffers than included in the kit. They may be purchased separately.
- Add 10 µL ApoH magnetic beads per sample, unless sample exceeds 10 mL. If so, increasing bead volume may be needed.
- Samples exceeding 20 mL may be agitated by orbital agitation (set a wheel to 3 rpm) instead of a 1000 rpm up-right agitation.
- Large samples take time to reach the right temperature. Make sure the sample temperature is below 10°C before adding the beads.

Handling beads and buffers

- Open the Peps6 magnetic beads vial in a sterile environment: contaminations will reduce stability and impair efficiency.
- Always add the Peps6 magnetic beads into the sample, not vice-versa.
- According to the micro-organism or the sample, the choice and the quantity of capture buffer may be optimized. For example, some viruses do not require the presence of the Additive FS. This additive must be left out when performing cell infection after the capture procedure.
- Use sterile osmosed water for buffer dilution. Check that Buffer Buffer TAS and Additive FS are indeed **1X concentrated** when mixed in the sample. The combination of Buffer TAS and liquid Additive FS (named Binding Buffer) should be used immediately; do not store for future use.
- The liquid form of Additive FS (after resuspension in Solvent FS) is a clear liquid that will turn light yellow when improperly stored. Discard yellowish Additive which reduces capture efficiency and use a new Additive FS aliquot.
- Strictly follow the Additive FS guidelines for resuspension. Incorrect (short) resuspension will lead to sub-optimal results. Do not heat!

Incubation

- Respect temperature and time for incubation to ensure best results. Do not exceed 10°C.
- Choose a test tube big enough to ensure correct agitation, for example: use a 1.5 mL tube for a 1 mL reaction.
- Tubes should be kept up-right (small and medium tubes) with vigorous agitation so the beads remain in suspension. For example, set a Thermomixer to 1000 rpm.
- Use glass or polypropylene plastic tubes only, avoid polystyrene.

Magnetization

- Increase magnetization time if some beads remain in the supernatant or if the bead pellet is disrupted by the pipet tip. Usually, this step ranges from 2 min (for cell culture supernatant) to 6 min (for whole blood).
- Use high energy neodymium magnets (8-12 kg attraction force) which insure the complete magnetization of beads. Low force magnets will lead to bead and micro-organism loss. Too strong magnets may embed the beads in the plastic tube.
- Remove floating bubbles before aspirating the supernatant.
- Do not let the beads magnetize over 30 min. Virus integrity may be damaged.

Wash

- Washing is not needed for cell supernatant, blood plasma or serum unless the detection system is very sensitive. Complex samples like whole blood may require 2 washes of the bead pellet. Wash on magnet. Never vortex beads in the wash solution.
- Buffer TAS 1X may be replaced by another wash buffer. Contact us to check its compatibility with the procedure.

Detection

- When applicable, the lysis step is crucial to reach successful micro-organism detection. Efficiency of lysis buffer depends greatly on chemical formulation and may differ from one supplier to another. Add a lysis control if possible to check the efficiency. Don't hesitate to harshly vortex the Peps6 beads in the lysis buffer. Incubate at 37°C instead of room temperature if room temperature is recommended for lysis.
- If an optical density measurement is needed, remove the beads with a magnet and test the supernatant only. The beads are dark brown and will interfere greatly with optical measurements.

12 – BIBLIOGRAPHY

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