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

Increasing sensitivity, improving diagnostics
www.apohtech.com

S.A. of 729 885 € capital
94, Allée des Fauvettes, 34280 La Grande Motte, FRANCE
Email: info@apohtech.com – Phone: +33 (0)4 11 75 94 85
Mobile: +33 (0)6 50 60 33 17 – Fax: +33 (0)4 11 75 95 46
R.C.S. Montpellier/SIRET: 429 750 839/00038 – APE: 7219Z
VAT: FR18429750839

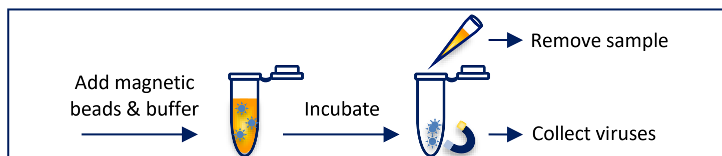
1 – INTRODUCTION

The Peps6-CaptoVIR kit is an **innovative sample pretreatment** tool for the **isolation of viruses** from any biological sample. Isolated and concentrated viruses are then easier to identify/detect with higher **sensitivity**.

The **synthetic** molecule called Peps6 is derived from the human ApoH protein, also known as Apolipoprotein H or Beta-2 glycoprotein 1, a protein able to bind micro-organisms including **viruses** (1-2), fungi (3) and bacteria (4-6). This **multiplex 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 with increased sensitivity (7-10). The capture buffer provided in the kit targets viral isolation, by increasing the viral affinity of the Peps6.

2 – PRINCIPLE

Peps6 is bound to magnetic beads, that are added to the sample previously diluted in the provided capture buffer. The viruses are captured on the Peps6 beads during a short incubation step. Bead-bound viruses are collected in the test tube using a magnet while the initial sample and its potential inhibitors are removed. Viruses are then ready to be processed by usual methods such as molecular techniques (PCR), immunological detection (ELISA, WB) or culture in appropriate cells.



3 – REAGENTS

REF MP20006 – Peps6 magnetic beads
About 10^{13} beads/mL of Peps6-coated magnetic particles (~200 nm diameter) are in a buffer containing < 0.02% sodium azide.

REF TP10002 – Buffer TAS 20X
The Buffer TAS is a clear binding buffer concentrated 20X. Dilute before use.

REF TP10007 – Additive FS 100X
Additive FS is supplied as a light-sensitive powder to be diluted with Solvent FS 100X. Dilute before use.

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.

4 – STORAGE

- Store at 2-8°C upon reception. Keep reagents sterile.
- 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.
- Opened 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. Therefore, the **liquid form** of Additive FS must be stored away from light at -20°C, where it remains stable until the expiration date.
- Do not store Buffer TAS if Additive FS has been added, use immediately.
- The Peps6 magnetic beads vial should be stored upright to always keep beads within their storage solution.

5 – MATERIAL REQUIRED, NOT PROVIDED

- Well preserved samples, up to 2 mL volume/sample.
- Laminar flow hood or any particular microbiologic environment required by the type of micro-organism targeted.
- 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.
- Lateral attraction magnet, compatible with the test tube.
- Materials and reagents required for processing the isolated viruses (for lysis, culture, identification or revelation).

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 type of virus and its use once captured (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 provides general guidelines for the isolation of viruses in **up to 2 mL samples**. Further optimization may be required for higher sample volumes or for specific samples or for specific viruses. Please contact our technical support:

info@apohtech.com

The Peps6 capture mechanism differs from regular antibody-antigen interactions. Thus, good sample preservation is paramount. Indeed, viruses damaged by inactivation or repeated freeze-thaw cycles may lose their affinity to the Peps6 molecule, leading to reduced viral isolation.

- Sample volume may be scaled up or down. Scale up sample volume if low viral titers are suspected.
- Do not add FS additive when capturing ISAV virus (Orthomyxoviridae) or when infecting cells with Peps6-bound viruses.
- Add FS additive for other viruses or when sample also contains bacteria.

8 – INSTRUCTIONS FOR USE

First use: dilute Additive FS

Add Solvent FS 100X into the Additive FS 100X, the required volume is noted on the label. 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. The Additive FS is now in liquid form, still at 100X concentration. It should be aliquoted in several tubes for future use.

First use: dilute Buffer TAS

Dilute Buffer TAS from 20X to 1X concentration in sterile osmosed water. For example, 1 mL TAS 20X + 19 mL water = 20 mL TAS 1X. Vortex.

Capture

- Dispense up to 2 mL sample in a capture tube. Measure volume.
- Add 4 volumes of TAS 1X.
- Add 0.05 volumes Additive FS 100X (optional).
- Vortex sample diluted in capture buffer.
- Vortex Peps6 magnetic beads and add 10 µL beads.
- Agitate gently, 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 an Eppendorf 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.** Do not let the pellet dry.

Example:
1 mL sample + 4 mL TAS 1X + 50 µL FS 100X + 10 µL Peps6 beads

Washing (optional)

Washing is not needed for cell supernatant, blood plasma or serum. Some samples like whole blood may require washes, depending on the compatibility of the detection method.

- Gently add 1 mL of Buffer TAS diluted 1X on the beads pelleted on the magnet. Don't suspend the beads if possible.
- Discard supernatant without disturbing the pellet of Peps6 magnetic beads.
- Repeat washing procedure once if needed.

9 – 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: If beads are resuspended in 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.

10 – TROUBLESHOOTING

Some guidelines are given below. Please contact our technical support for any remaining questions:

info@apohtech.com

Sample collection

- Use preferably fresh samples or samples frozen at -20/-80°C shortly after their draw. Repeated freeze-thaw cycles of samples should be avoided.
- Never use inactivated viruses. Capture efficiency decreases drastically after heat inactivation and less after inactivation by irradiation.

IF SOLID sample:

- Grind your solid sample (ex: meat, tissue) in TAS buffer diluted to 1X then remove clumps by spinning or filtering the sample on a sterile gauze. Perform virus isolation on supernatant or filtrate.
- Adding 3% CTAB (cetyltrimethyl ammonium bromide) in the grinding step may optimize virus release from cells. The percentage should be adjusted according to your sample. Contact our technical support to check the compatibility of other sample lysis buffers.

IF BLOOD-derived sample:

- Avoid pooling plasma, serum or whole blood which may produce a coagulum that can trap magnetic beads. Bead aggregation leads to reduced viral isolation.
- Work with samples from EDTA anti-coagulant blood collection tubes.

Handling samples

- The reaction volume is usually 5 times the sample volume. Choose a test tube big enough to ensure correct agitation, for example: use a 1.5 mL tube for a 1 mL reaction.
- Increase sample dilution to 10 times if detergents are present, such as 0.1% SDS or 3% CTAB.
- All TAS diluted samples should be rapidly put in contact with the Peps6 magnetic beads.
- Large sample volumes (2-100 mL) require more beads and buffers than included in the kit. They may be purchased separately.
- Large samples take time to reach the right temperature. Make sure the sample temperature is below 10°C before adding the beads.

Handling beads

- The Peps6 magnetic beads **must not be** 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.
- Open the Peps6 magnetic beads vial in a sterile environment: contaminations will reduce stability and impair efficiency.
- 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.
- Add 10 µL Peps6 magnetic beads per sample, unless sample exceeds 10 mL. If so, increasing bead volume may be needed.
- Be careful when opening tubes to ensure no spraying of the beads from the lid.

Handling buffers

- Check if FS additive should be used: necessary for most viruses and for bacteria-containing samples but not if cell infection is wanted or for ISAV virus isolation (Orthomyxoviridae).
- Use sterile osmosed water for buffer dilution.
- Strictly follow the Additive FS guidelines for resuspension. Incorrect (short) resuspension will lead to sub-optimal results. Do not heat!
- Diluted Additive 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. Discard remaining FS-containing TAS buffer as it is not stable.
- All reagents included in the kit are also available separately.

Incubation

- Use glass or polypropylene plastic tubes only, avoid polystyrene.
- Respect temperature and time for incubation to ensure best results. Do not exceed 10°C.
- If some beads pellet during the incubation, increase agitation.
- If some beads enter the lid during incubation, spin the tube for 3 sec before setting on the magnet stand. Beads in the lid may splash when opening the tube, leading to virus loss and contamination.
- Samples exceeding 20 mL may be agitated by orbital agitation (set a wheel to 3 rpm) instead of a 1000 rpm up-right agitation.

Magnetization

Please note that attraction efficiency and speed vary between different commercially-available magnets.

- 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.
- 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 (if cell culture supernatant) to 6 min (if whole blood). Do not let the beads magnetize over 30 min. Virus integrity may be damaged.
- Remove floating bubbles before aspirating the supernatant.

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. Gentle pipetting is recommended to increase washing efficiency.
- 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.

11 – BIBLIOGRAPHY

1. Stefas E et al. Human plasmatic apolipoprotein H binds human immunodeficiency virus type 1 and type 2 proteins. AIDS Res Hum Retroviruses 1997, 13(1):97-104.
2. Stefas I et al. Hepatitis B virus Dane particles bind to human plasma apolipoprotein H. Hepatology 2001, 33(1):207-17
3. Calvino JR et al. Use of magnetic nanoparticles for the specific separation and the molecular detection of micro-organisms on whole blood. ePoster for the 2015 ECCMID, Copenhagen, Denmark
4. Zhang L et al. Staphylococcus aureus expresses a cell surface protein that binds both IgG and beta2-glycoprotein I. Microbiology 1999, 145 (Pt1):177-83.
5. Agar C et al. β2-glycoprotein I: a novel component of innate immunity. Blood 2011, 117(25):6939-47.
6. Bouma B. et al. Adhesion mechanism of human b2-glycoprotein I to phospholipids based on its crystal structure, The EMBO Journal 1999, 18 (19): 5166-5174.
7. Veas F et al. Apolipoprotein H, an acute phase protein, a performing tool for ultra-Sensitive detection and isolation of microorganisms from different origins. Ch. 2 pages 21-42 in « Acute phase proteins as early non-specfic biomarkers of Human and veterinary diseases » 408 pages. Edited by Francisco Veas, 2011. Publisher InTech, Vienna, Austria and Rijeka, Croatia.
8. Adlhoch C et al. Highly sensitive detection of the group A Rotavirus using Apolipoprotein H-coated ELISA plates compared to quantitative real-time PCR. Virology Journal 2011, 8:63.
9. Stefas I et al. Interactions between Hepatitis C Virus and the Human Apolipoprotein H Acute Phase Protein: A Tool for a Sensitive Detection of the Virus. PlosOne 2015, Oct 26 (10):1-24.
10. Vutukuru MR et al. A rapid, highly sensitive and culture-free detection of pathogens from blood by positive enrichment. J Microbiol Methods. 2016 Dec; 131:105-109. doi: 10.1016/j.mimet.2016.10.008. Epub 2016 Oct 17.