ApoH-CaptoVIR kit

Reference: MP10022

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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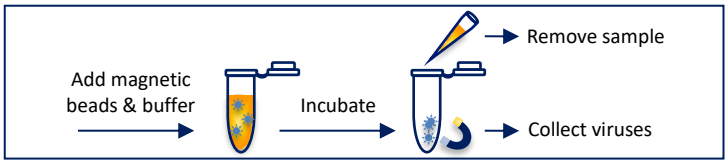
1 – INTRODUCTION

The ApoH-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**.

ApoH, also known as Apolipoprotein H or Beta-2 glycoprotein 1, is a human 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 ApoH protein.

2 – PRINCIPLE

ApoH is bound to magnetic beads, that are added to the sample previously diluted in the provided capture buffer. The viruses are captured on the ApoH 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 MP20001 – ApoH magnetic beads
About 10^{13} beads/mL of ApoH-coated magnetic particles (~200 nm diameter) are in a buffer containing < 0.02% sodium azide.

REF TP10002 – Buffer TAS 20X (2-8°C)
The Buffer TAS is a clear binding buffer concentrated 20X. Dilute before use.

REF TP10007 – Additive FS 100X (2-8°C solid, -20°C solubilized)
Additive FS is supplied as a light-sensitive powder to be diluted with Solvent FS 100X. Dilute before use.

REF TP10008 – Solvent FS 100X (2-8°C)
Solvent FS is an aqueous solution for the resuspension of Additive FS concentrated 100X. Do not dilute before adding to Additive FS.

REF TP10011 – Buffer CV-1 20X (15-25°C)
Buffer CV-1 is a clear binding buffer concentrated 20X. It should be at 1X final for the capture reaction.

REF TP10013 – Buffer GV-2 20X (15-25°C)
Buffer GV-2 is a clear binding buffer concentrated 20X. It should be at 1X final for the capture reaction.

4 – STORAGE

- Store requested reagents 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 Additive FS once it has been resuspended which must be stored at -20°C and Buffer CV-1 that must be stored at room temperature.
- 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.
- Buffer CV-1 can crystallize when stored at low temperature. If white crystals appear, warm Buffer CV-1 bottle at 37°C until dissolution before use.
- The ApoH magnetic beads vial should be stored upright to always keep beads within their storage solution.

5 – MATERIAL REQUIRED; NOT PROVIDED

- Well preserved samples.
- 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 ApoH 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**: whole blood, plasma/serum, solids such as food or tissue, transport media and oral fluids such as saliva, sputum, mouthwashes or bronchoalveolar lavage**. Further optimization may be required for high sample volumes or other specific samples or for specific viruses. Please contact our technical support:

info@apohtech.com

The ApoH 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 ApoH molecule, leading to reduced viral isolation.

- Sample volume is adjustable. Scale up sample volume if low viral titers are suspected.
- Do not add FS additive when infecting cells with ApoH-bound viruses.

8 – INSTRUCTIONS FOR USE

Before capture

- 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.

- Buffer CV-1 can crystallize when stored at low temperature. If white crystals appear, warm Buffer CV-1 bottle at 37°C until dissolution before use.
- Set a thermomixer at 4°C or place your agitating device in a fridge

Capture will differ depending on the type of sample that is treated. Please refer to the following table to access the rightful protocol:

Sample	Protocol	Needed buffers
Blood derived samples	**A**	TAS + FS
Solids (food/tissue)	**B**	CV-1
Transport media	**C**	CV-1
Oral fluids	**D**	CV-1 + GV-2

A. Capture from blood derived samples: whole blood, serum or plasma

Note: *For whole blood, make sure that the sample was stored for max. 24h at 4°C after withdrawal. Do not use whole blood that has been frozen or kept over 24h at 4°C. For serum/plasma, collect the plasma/serum within 24h of blood withdrawal. Plasma/serum can be tested after being stored at -20°C. Please note that a decrease in capture is observed for serum/plasma samples that have been stored for several years at -20°C.*

Requested buffers: TAS 20X and Additive FS 100X suspended in Solvent FS.

Dilution factor: 1/5

Sample preparation

- 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.
- Vortex the sample diluted in capture buffer.
- Briefly vortex ApoH magnetic beads and add 10 µL of 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 ApoH magnetic beads remain in suspension. For example, set an Eppendorf Thermomixer to 1000 rpm.
- Place the reaction tube on a magnet until all ApoH magnetic beads are laterally pelleted and the supernatant has cleared up (wait at least 5 min, especially for whole blood where the pellet cannot be seen).
- Discard supernatant without disturbing the pellet of ApoH magnetic beads. **Viruses are now concentrated in the pellet.** Do not let the pellet dry.

Example for 400 µL of sample (2 mL final volume):
400 µL sample + 1.6 mL TAS 20X+ 20 µL FS 100X+ 10 µL ApoH beads
Example for 1 mL of sample (5 mL final volume):
1 mL sample + 4 mL TAS 1X + 50 µL FS 100X + 10 µL ApoH beads

B. Capture from solids (food or tissue)

Requested buffer: CV-1 20X

Sample preparation

- Prepare a 1X solution of CV1 buffer by diluting the buffer 20 times.
- Use this 1X solution to grind your solid sample (ex: food, tissue).
- Remove clumps by spinning or filtering the sample on a sterile gauze. Perform viral capture on supernatant or filtrate.

Here, 10 µL of beads should be added per 10 mL of sample.

- Homogenize gently by inverting the tube.
- Incubate for 5 min at 2-8°C under proper agitation: tubes should be kept with vigorous agitation so the magnetic beads remain in suspension. For example, set an Eppendorf Thermomixer to 750 - 1000 rpm or use a rotating wheel.
- Place the reaction tube 10 min on a magnet or until all magnetic beads are laterally pelleted and the supernatant has cleared up, the larger the volume, the longer it will take.
- Discard supernatant without disturbing the pellet of magnetic beads. **Viruses are now concentrated in the pellet.** No washing is needed prior to viral detection. Do not let the pellet dry.

C. Capture from transport media

Note: Make sure that transport media doesn't contain inactivation compounds.

Requested buffer: CV-1 20X

Dilution factor: 1/10

Sample preparation

Universal or Viral Transport Media should be diluted 10 times for

optimal capture, please note that capture should be performed in the adequate tubes. CV-1 must be at a 1X final concentration.
Prepare the tubes according to this table:

Reagents	Volumes requested				
Transport Media	**100 µL**	**500 µL**	**1 mL**	**2 mL**	**3 mL**
Requested tube	*1.5 mL*	*5 or 15*	*15 mL*	*50 mL*	*50 mL*
CV-1 20X	50 µL	250 µL	500 µL	1 mL	1.5 mL
Sterile H2O	850 µL	4.25 mL	8.5 mL	17 mL	25.5 mL
Final volume	1 mL	5 mL	10 mL	20 mL	30 mL
Beads (µL)	10	10	10	20	30

Here, 10 µL of beads should be added per 10 mL of sample.
- Homogenize gently by inverting the tube.
- Incubate for 5 min at 2-8°C under proper agitation: tubes should be kept with vigorous agitation so the magnetic beads remain in suspension. For example, set an Eppendorf Thermomixer to 750 - 1000 rpm or use a rotating wheel.
- Place the reaction tube 10 min on a magnet or until all magnetic beads are laterally pelleted and the supernatant has cleared up, the larger the volume, the longer it will take.
- Discard supernatant without disturbing the pellet of magnetic beads. **Viruses are now concentrated in the pellet.** No washing is needed prior to viral detection. Do not let the pellet dry.

D. Capture from oral fluids

Requested buffers: CV-1 20X and GV-2 20X
Dilution factor: none or 1:1

Sample preparation

Dilution of the sample should be done according to the thickness of the sample. For example, mouthwashes performed with water or saline solution do not need any dilution, but thick saliva samples or sputum should be diluted at least in a 1:1 ratio with a 2X buffer solution in sterile water. This will allow optimal diffusion of the magnetic beads. CV-1 and GV -2 20X buffers should always be at 1X final concentration.
Note: Mucus can sometimes precipitate in the capture buffer. Discard the precipitate with the pipette before proceeding to capture. It will avoid the beads getting stuck in the precipitate, allowing them to diffuse evenly.

- **For mouthwash, bronchoalveolar lavage samples/already highly diluted samples**:

- Calculate the volume of 20X buffers you should add by dividing your desired final volume by 20. Add your buffers to the tube and complete with your sample up to your desired final volume. Vortex.

Example for 1 mL:
50 µL CV-1 20X + 50 µL GV-2 20X + 900 µL sample
Example for 2 mL:
100 µL CV-1 20X + 100 µL GV-2 20X + 1.8 mL sample

- **For thicker samples such as saliva or sputum (rich in mucus)**:
Prepare a **2X Capture Buffer mastermix** solution. Define the volume needed. Divide this volume by 10 to obtain the volume of 20X buffers needed (dilution to the 10th of the buffers). Add them to the tube, complete to the desired final volume with sterile water. Vortex.
- Mix one volume of sample to one volume of 2X Capture buffer (1:1 ratio) in a tube. Vortex.

Example for 10 mL of 2X Capture buffer mastermix:
1 mL CV-1 20X + 9 mL sterile water
Capture for 1 mL: 1 mL sample + 1 mL 2X buffer = 2 mL final
Capture for 2 mL: 2 mL sample + 2 mL 2X buffer = 4 mL final

Samples are ready for capture.
Here, 10 µL of beads should be added per mL of **sample**.
- Homogenize gently by inverting the tube.
- Incubate for 5 min at 2-8°C under proper agitation: tubes should be kept with vigorous agitation so the magnetic beads remain in suspension. For example, set an Eppendorf Thermomixer to 750 - 1000 rpm or use a rotating wheel.
- Place the reaction tube 5 min on a magnet or until all magnetic beads are laterally pelleted and the supernatant has cleared up, the larger the volume, the longer it will take.
- Discard supernatant without disturbing the pellet of magnetic beads. **Viruses are now concentrated in the pellet.** No washing is needed prior to viral detection. Do not let the pellet dry.

9 – VIRAL DETECTION

The bound viruses can be revealed directly on the ApoH 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 ApoH 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 ApoH magnetic beads on a magnet. Transfer the supernatant in a new tube. Proceed with your usual DNA/RNA extraction and (RT)-PCR protocol.

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

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

Other: Resuspend the ApoH 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 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
- Check the reaction volume. Choose a test tube big enough to ensure correct agitation, for example: use a 1.5 mL tube for a 1 mL reaction.

- Large sample volumes (2-50 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 ApoH 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 ApoH 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 ApoH magnetic beads per sample, unless sample exceeds 10 mL, or if . 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 ApoH 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.

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