Peps6-CaptoBAC kit
Reference: MP10031

For research use only

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

ApoH technologies

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-CaptoBAC kit an **innovative** sample pretreatment tool for bacteria isolation before their **sensitive** identification/detection.

2 – PRINCIPLE

In the Peps6-CaptoBAC kit, Peps6 is bound to magnetic beads. The kit is supplied with a special binding buffer that increases the affinity of the Peps6 towards bacteria. 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 bacteria, linked via Peps6 to the magnetic beads, are kept in the test tube using a magnet. Bacteria 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 media.

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 TP10004 – Buffer TTGB 10X
The Buffer TTGB 10X is a light yellow aqueous binding buffer filtered at 0.2 µm and concentrated 10X. Dilute according to the instructions below.

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 reagents remain stable at 2-8°C until the expiration date.
- The Peps6 magnetic beads vial should be stored upright to always keep beads within their storage solution.
- After use, all reagents should be rapidly stored at 2-8°C.

5 – MATERIAL REQUIRED, NOT PROVIDED

- Sterile osmosed water.
- Suitable micropipettes and sterile 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 bacteria in up to 5 mL samples. Further optimization may be required in order to achieve optimal binding capacity depending on the bacterial strain, sample nature and volume.
The mechanism of ApoH 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 bacteria capture. Same care should be taken after capture if retaining viable micro-organisms is an issue.
- Increase bead volume only if high micro-organism loads are suspected. Beads are able to bind high numbers of micro-organisms: 1 µL of beads binds 1E+7 *E. coli* from a pure culture.

8 – SAMPLE COLLECTION AND HANDLING

Our current data show that the Peps6 magnetic beads can capture bacteria in all kinds of solid (after suspension) or liquid samples.
- Grind your solid sample (ex: meat, tissue) in capture buffer diluted to 1X. Then filter the mix on a sterile gauze before adding the beads, otherwise the beads may get caught in sample bits and won't be able to get magnetized.
- 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.
- In the case of bacteria spiking, use **clinical strains** not **"collection" bacteria** (as ATCC strain = American Type Culture Collection). Indeed, many collection bacteria lose their attraction to the ApoH protein or the ApoH-derived Peps6 molecule.
- When using whole blood, 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 micro-organism titers are suspected. For volumes above 5 mL, contact our technical support.

Damaged micro-organisms may lose their affinity to the ApoH protein or the ApoH-derived Peps6 molecule, so:
- Use preferably fresh material.
- Check the viability of the bacteria in frozen samples. Repeated freeze-thaw cycles of samples should be avoided.
- Use of poor-quality starting material leads to reduced sensitivity.

9 – INSTRUCTIONS FOR USE

Sample dilution in capture buffer
Dilute sample in capture buffer then vortex. Final buffer concentration must be 1X after sample dilution:
- Small size sample (1-199 µL): dilute Buffer TTGB 10X to 1X concentration in sterile osmosed water. Add enough 1X Buffer TTGB in sample to reach 1 mL total volume.
- Large size sample (0.2-5.0 mL): dilute Buffer TTGB 10X to 2X concentration in sterile osmosed water. Add 1 volume of Buffer TTGB 2X to 1 volume of sample.

Capture
- Prior to use, thoroughly resuspend the Peps6 magnetic beads by gentle pipetting or manual inversion of the vial (do not vortex).
- Add 20 µL Peps6 magnetic beads per sample.
- Gently homogenize (do not vortex).
- Incubate for 30 min at 35-37°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. **Bacteria are now concentrated in the pellet.**

Washing (optional)
Washing is not needed for pure culture, blood plasma or serum. Complex samples like whole blood may require washes:
- Gently add 1 mL of PBS (without Ca^{2+}/Mg^{2+}) 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 – BACTERIA DETECTION

The bound bacteria 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.

Culture: Resuspend the Peps6 magnetic beads in the appropriate media. Strike directly the suspension in a Petri dish. Incubate at the bacteria's preferred temperature.

PCR: Resuspend the Peps6 magnetic beads in your lysis buffer. Vortex vigorously during 15 seconds to disrupt bead pellet. After the lysis step, 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 extraction and 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.

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

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.
- Use sterile osmosed water for buffer dilution. Check that Buffer TTGB is indeed **1X concentrated** when mixed in the sample.
- Check that Buffer TTGB is not contaminated.
- According to the micro-organism or the sample, the choice and the quantity of capture buffer may be optimized.

Large sample volumes

- Large sample volumes (5-100 mL) require more beads and buffers than included in the kit. They may be purchased separately.
- Sample dilution in capture buffer: add 2X or 10X concentrated Buffer TTGB directly into the sample to reach 1X final buffer concentration.
- Add 20 µL Peps6 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. Let the sample reach either room temperature or the incubation temperature before adding the beads.

Incubation

- Respect temperature and time for incubation to ensure best results.
- 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) to 15 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. Micro-organism 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.
- PBS may be replaced by another wash buffer. Contact our technical support to check its compatibility with the procedure.

Detection

- Some bacterial species are not able to grow after capture. These bacteria are in a viable but not cultivable state. Check the capture by microscopy or PCR.
- 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.
- 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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