Peps6 magnetic beads

Reference: MP20006

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 magnetic beads an **innovative** sample pretreatment tool for micro-organism isolation before their **sensitive** identification/detection.

2 – PRINCIPLE

The Peps6 synthetic molecule, coated on magnetic beads, captures micro-organisms present in the sample of interest. Binding buffers that increase the affinity of the Peps6 towards specific micro-organisms are available separately. The initial sample and its potential inhibitors can be removed whereas the captured micro-organisms, linked via Peps6 to the magnetic beads, are kept in the test tube using a magnet. Micro-organisms 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/ cells.

3 – REAGENTS

REF MP20006 – Peps6 magnetic beads

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

4 – STORAGE

- The Peps6 magnetic beads may travel at ambient temperature without altering its function; store at 2-8°C upon reception.
- The Peps6 magnetic beads vial should be stored upright to always keep beads within their storage solution.
- The beads remain stable at 2-8°C until the expiration date.
- After use, rapidly store the Peps6 magnetic beads at 2-8°C.

5 – MATERIAL REQUIRED, NOT PROVIDED

- Suitable micropipettes and sterile filter tips.
- Suitable reaction tubes, glass or plastic (polypropylene only, avoid polystyrene).
- Suitable equipment for the sample agitation during incubation.
- Sample dilution buffer (optional): a panel of ApoH-Technologies Buffers is available. Their dilution requires sterile osmosed water.
- 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 micro-organisms. Further optimization may be required in order to achieve optimal binding capacity depending on the micro-organism type and sample nature.
The mechanism of ApoH capture **differs** from regular antibody-antigen interactions. To ensure **better success** in your trials, contact our technical support**:**

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- The Peps6 magnetic beads **must not be vortexed**, frozen, dried, handled at high temperatures (> 60°C) or extreme pH (>9 or <5), prior to micro-organism 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 or 1E+7 Ebola viruses in a clinical plasma sample.

8 – SAMPLE COLLECTION AND HANDLING

Our current data show that the Peps6 magnetic beads can capture micro-organisms in all kinds of solid (after suspension) or liquid samples.
- Grind your solid sample (ex: meat, tissue) in PBS or an ApoH binding buffer. 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.
- 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**.
- Scale up sample volume if low micro-organism titers are suspected.
- All diluted or treated samples should be rapidly put in contact with the Peps6 magnetic beads.

Damaged micro-organisms 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.
- Check the viability of the bacteria in frozen samples.
- Never use **inactivated viruses**.
- Use of poor-quality starting material leads to reduced sensitivity.

9 – INSTRUCTIONS FOR USE

Sample dilution in capture buffer (optional)
Dilute sample according to the recommended protocol for each specific buffer. Buffer TAS is optimal for viruses and Buffer TTGB is optimal for bacteria.

Capture
- For large samples, make sure the sample reaches the incubation temperature before adding the beads.
- For small samples, dilute in PBS or appropriate solution to obtain at least 400 µL in order to insure correct agitation.
- Prior to use, thoroughly resuspend the ApoH magnetic beads by gentle pipetting or manual inversion of the vial (do not vortex).
- Add 10-20 µL Peps6 magnetic beads per sample, unless sample exceeds 20 mL. If so, increasing bead volume may be needed.
- Gently homogenize (do not vortex).
- Incubate for 30 min at 35-37°C for bacteria or 2-8°C for viruses 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. Samples exceeding 20 mL may be agitated by orbital agitation (set a wheel to 3 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. **Micro-organisms 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 for viruses or PBS (without Ca^{2+}/Mg^{2+}) for bacteria 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 – MICRO-ORGANISM DETECTION

The bound micro-organisms 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.
- For bacteria, strike directly the suspension in a Petri dish. Incubate at the bacteria's preferred temperature. Note that the presence of beads may interfere with bacteria growth for some species, if so, check micro-organism presence by PCR.
- For viruses, add directly the suspension in a compatible cell culture.

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.

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:

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Samples

To optimize the availability of viruses (not bacteria) 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.

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 if needed.
- According to the micro-organism or the sample, the choice and the quantity of capture buffer may be optimized.

Incubation

- Respect temperature and time for incubation to ensure best results**.**
- Large samples take time to reach the right temperature. Let the sample reach the incubation temperature before adding the beads.
- Choose a test tube big enough to ensure correct agitation, for example: use a 1.5 mL tube for a 1 mL reaction.
- Use glass or polypropylene plastic tubes only, avoid polystyrene.
- 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. For large volumes, use a 3 rpm orbital agitation on a wheel.

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 6-15 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 or Buffer TAS 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.

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