ApoH microplate

Reference: PQ09011

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

ApoH is a plasmatic protein able 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).

The ApoH microplate is therefore a very innovative and versatile tool for micro-organism detection from complex biological samples. It is provided as a convenient 96-well format divided in 12 strips of 8 wells that may be used independently.

2 – PRINCIPLE

The ApoH microplate may be used in all standard sandwich ELISA protocols, where the ApoH protein replaces the fixed capture antibody allowing a much wider range of targets. Binding buffers that increase the affinity of the ApoH towards specific micro-organism antigens are available separately.
The initial sample and its potential inhibitors can be removed whereas the captured micro-organisms, linked via ApoH to the microplate, can be washed to eliminate unbound material. Micro-organisms are then ready to be processed by immunological detection (ELISA) or culture in appropriate media. The client antibodies used will guarantee the specificity of the identification/detection of the retained micro-organisms.

3 – REAGENTS

REF PQ09011 – ApoH microplate
Microplate of 96 modulable wells (12 strips of 8 wells) coated with ApoH and packed in a sealed aluminum bag with a desiccant.

Note: Binding Buffer TT is available separately.

4 – STORAGE

- The ApoH microplate may travel at ambient temperature without altering its function; store at 2-8°C upon reception.
- The ApoH microplate stable at 2-8°C until the expiration date.
- Place the unused strips of the ApoH-coated microplate in the original bag, seal tightly and keep them at 2-8°C.

5 – MATERIAL REQUIRED, NOT PROVIDED

- Suitable micropipettes, filter tips and reaction tubes.
- Suitable equipment for the sample agitation during incubation.
- Sample dilution and wash buffer: Buffer TT is recommended, alternatively use another buffer from ApoH-Technologies or PBS or Tris buffer. ApoH-Technologies buffers are concentrated and must be diluted in sterile osmosed water prior to use.
- Incubator regulated at the appropriate temperature.
- Materials and reagents required for the revelation of targeted micro-organisms.

6 – SAFETY AND PRECAUTIONS

- For better stability, handle with care to **avoid any contaminations**.

- The ApoH microplate contains < 0.01% of thimerosal. Traces of thimerosal do not interfere with ApoH capture, nor with micro-organism viability: there is no need to wash the plate prior to use.
- The ApoH is a protein of human origin. Although this protein is purified from plasma or serum, free of infectious agents (HIV, HBV, HCV) according to European regulations at the time of their collection, it is recommended to manipulate the ApoH magnetic beads as a potentially infectious product.
- 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 ApoH microplate **must not be** 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.
- Sample dilution in an appropriate buffer will enhance micro-organism capture. Buffer TT is recommended.

8 – SAMPLE COLLECTION AND HANDLING

Our current data show that the ApoH magnetic beads can capture micro-organisms in all kinds of solid (after suspension) or liquid samples.
- Use preferentially fresh samples and avoid pooling them.
- When using whole blood or plasma, choose the **EDTA anti-coagulant**.
- One ApoH microplate well holds an optimal working volume of 100 to 250 µL pure or diluted sample in a 300 µL well.
- Sample volume may be scaled up or down. Scale up sample if low micro-organism titers are suspected.
- All diluted or treated samples should be rapidly put in contact with the ApoH microplate.

Damaged micro-organisms may lose their affinity to the ApoH protein, 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

Sample dilution (optional)
- Dilute Buffer TT 20X to 1X concentration in sterile osmosed water. Vortex.
- Dilute sample 10 fold in diluted Buffer TT: 1 volume sample + 9 volumes 1X diluted Buffer TT.
- Vortex sample after dilution.

Sample dilution in Buffer TT will enhance micro-organism capture.

Binding
- Place the necessary number of ApoH strips on the plastic support.
- Add 100-250 µL pure or diluted sample to each well.
- Incubate for 90 minutes at 35 - 37°C.

Antigens are now immobilized on the ApoH microplate.

Washing
- Wash at least 3 times in 200 µL of appropriate wash buffer: Buffer TT or PBS is recommended.
- Wash buffer is eliminated after each washing step.

10 – MICRO-ORGANISM DETECTION

The bound micro-organisms can be revealed directly on the ApoH microplate using your standard protocols, which may be adapted if necessary.

ELISA: Use specific revelation antibodies for the targeted micro-organisms in your usual protocol.

Culture: Add an appropriate culture media directly in the ApoH microplate and incubate at the temperature suited to the target micro-organisms.

Other: 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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- Dilute sample 10 fold in 1X diluted Buffer TT. Alternatively, replace buffer TT by Buffer TAS or TTGB.
- Reduce sample/buffer dilution ratio.
- Respect temperature and time for incubation to ensure best results**.**
- If high background is visible, increase washing steps and check the eventual cross-reactivity of the revelation antibody to the ApoH protein.
- PBS or TBST may be replaced by another wash buffer. Contact our technical support to check its compatibility with the procedure.

12 – BIBLIOGRAPHY

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