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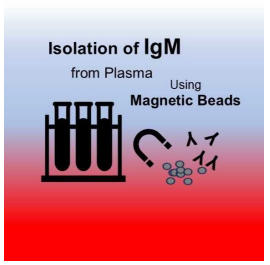
A simple and quick protocol for isolating human IgM antibodies from plasma using magnetic beads



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Protocol status: Working
We use this protocol and it's working

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Disclaimer

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Abstract

Magnetic beads are used in various applications, e.g. cell isolation, nucleic acid analysis and endotoxin elimination. Numerous groups are actively involved in the development of immunoglobulins for various research purposes. For example, many research projects require human IgM from human plasma. IgM are antibodies that appear in the first phase of infections with pathogens; their presence therefore indicates that the infection has only just begun. In addition, IgMs are often needed in research to determine their properties, and some groups are working to determine the properties of such antibodies for future therapeutic use. In all these cases, users need to isolate them from plasma.
We have developed a protocol to isolate such IgM antibodies using magnetic beads. You can also use this protocol to isolate sterile antibodies.

Image Attribution

Genekam IgM isolation magnetic beads

Guidelines

Ethical guidelines may apply, please check before starting experiments.

Materials

Equipment needed

- Magnetic beads (Genekam Magnetic beads antibody IgM isolation Kit, SB0215) sterile product - Tube A
- Magnetic rack (Genekam Magnetic separator for different tubes: 6 x 1.5 -2ml, SB0196)
- Buffer solution -Tube B
- Elution buffer - Tube C
- Stop solution - Tube D
- Pipettor
- Pipette tips
- **Lamina Flow if the user wants to produce a sterile product.**

Safety warnings

-Keep the magnetic beads away from sun light.
-If the package and the bottles are damaged don´t use the kit.
-Read the material safety data sheet.
-**If user wants to have sterile end product, one must work under laminar flow and with sterile instruments.**

Precautions

-The kit is intended for in vitro use only.
-The kit should only be used by trained persons.
-The user must read the manual for use carefully.
-The kit should not be used after the expiry date.
-The user should work very cleanly during the removal process.
-Decontaminate the instruments regularly (once a week).
-The user should wear protective gloves and laboratory clothing.

Ethics statement

As the user is working with human plasma, hence there may be ethics standards being applied here and may need of the approval from the ethics committee.

Before start

Please read manual.
Prepare the chemicals to be used.

1 1. Label the tubes and calculate the chemicals required.

2 The user must first wash the specified quantity of magnetic beads (Tube A) in 1000µl buffer solution (Tube B). For this purpose, 1000µl buffer solution is added to the beads, the tube is placed in the magnetic rack and the liquid is pipetted off after 2 minutes while the magnetic beads remain in the tube. Resuspend the beads in corresponding amount of buffer solution (30µl Tube B).

3 Performing of the isolation:
The user should use 30µl magnetic beads for the isolation of IgM from 2000µl plasma (or corresponding ratios). The user should use the plasma in a 1:4 dilution (add 1500µl buffer solution to 500µl plasma (Tube B).

4 Leave the mixture at room temperature for 30 minutes in dark. Shake occasionally.

5 Place the tube in the Genekam magnetic rack for 2 minutes. During this step, the magnetic beads are attracted towards the magnet.

6 Carefully remove the liquid with a pipette tip without distrubing the magnetic pellet and collect it in a new tube alternatively one can tilt the magnetic rack to drop the fluid in waste collecting container while keeping the magnetic pellet in the tube. (This is only possible, if user is using Genekam magnetic rack as the tubes fits very tightly in this rack.)

7 Wash twice with buffer solution:
To do this, add 1000µl of buffer solution (Tube B) to the beads, place the tube in the magnetic rack and pipette off the liquid after 2 minutes while the magnetic beads remain in the tube.

8 Elution with elution buffer:
To do this, add 100µl of elution buffer (Tube C) to the beads and incubate for 10 minutes. Place the tube in the magnetic rack and collect the liquid in a fresh sample tube after 2 minutes. Finally, add 25µl of stop solution (Tube D).

9 The isolation of the IgM antibodies should be confirmed using ELISA.

Protocol references

Bhatia, S. (2023). A Simple, Rapid, and Highly Sensitive Magnetic Beads ELISA for Detection of SARS CoV-2 Antibodies (IgG) in Human Plasma Samples as a Point of Care Assay. *Mikrobiolohichnyi Zhurnal*, *85*(6), 61-65. https://doi.org/10.15407/microbiolj85.06.061