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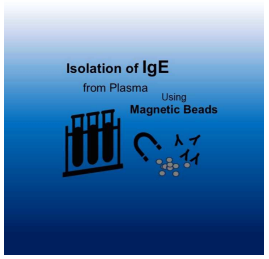
# A protocol for the isolation of IgE antibodies from human plasma using magnetic beads

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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

**A protocol for the isolation of IgE antibodies from human plasma using magnetic beads**

Human IgE antibodies are also present in plasma and there are many situations where isolation of these antibodies is essential. The concentration of such antibodies can vary in many allergic diseases.  Magnetic beads can be the material of choice to isolate such antibodies. The method is quite simple. It consists of 4 steps: 1. attachment of the antibodies to the magnetic beads 2. washing to remove the impurities 3. elution 4. neutralization of the solution containing the antibodies.

It is highly advisable to use magnetic racks that are designed to fit the microtubes exactly so that liquids can be removed without distrubing the bead pellet.

The advantage of the magnetic bead isolation method is that it is very fast and can be used for both small and large plasma volumes. Used plasma can be collected and used for further protein isolations. Used beads can be regenerated also.

We have developed a protocol for the isolation of IgE antibodies, which will be presented here.

If the user wants to isolate the antibodies for sterile purposes, he should work under laminar flow.

## Image Attribution

Genekam IgE magnetic beads

## Guidelines

Ethical guidelines may apply, please check before starting experiments.

## Materials

**Equipment needed**

- Magnetic beads antibody isolation kit (Human IgE; full kit including all buffers, SB0238)

- Magnetic beads (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

Since the user is working with human plasma, ethical standards may apply and ethics committee approval may be required.

## Before start

Please read manual.
Prepare the chemicals to be used.

1 **Preparatory work:**
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 IgE 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). It is also possible the cell cultures containing IgE to be used instead of plasma.

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 (Fig. 1, 2).

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 (Fig. 1). (This is only possible, if user is using Genekam magnetic rack as the tubes fits very tightly in this rack.)

7 **Two washing steps:**
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 step:**
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 isolated IgE antibodies should be confirmed using ELISA or other methods.

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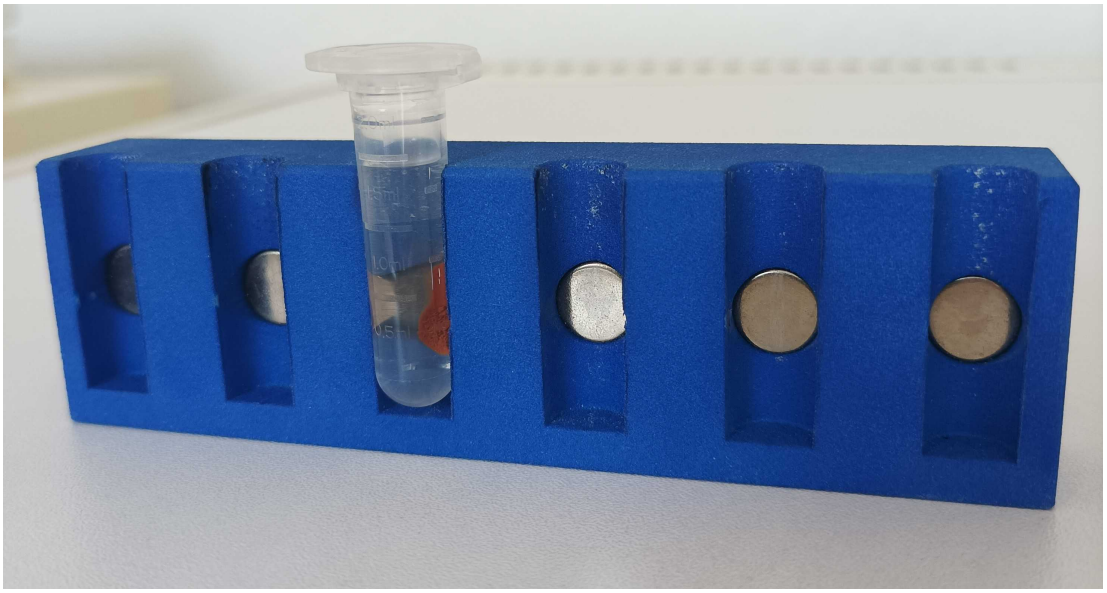

**Figure 1:** Tube with magnetic beads in the magnetic rack

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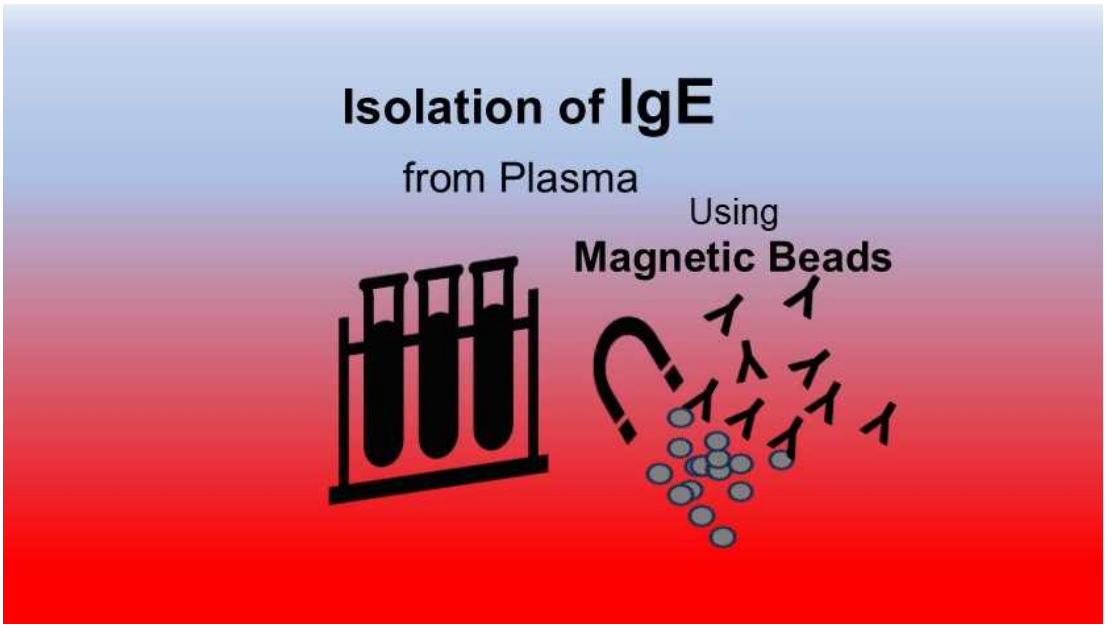


**Figure 2:** Scheme of antibody isolation with magnetic beads

## Protocol references

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