

Lot.
Ref. SB0149

MANUAL

Expiry Date: 1 year
Store at 4⁰C

To be used through technical person

This kit can be used to isolate the T-cells (CD 4 positive) from different sources like blood, bone marrow biopsies, cell cultures etc. It is based on CD4 biomarker. Magnetic beads will attach on the targeted cells through these biomarkers and they will be pulled out with the magnetic field. These can be used for different purposes e.g. to do the molecular testing of the presence of different molecular markers on the cells after isolation of RNA / DNA, development of vaccines, they can be cultured to test the drug sensitivities and can be used for microscopic, flow cytometric as well as fluorescence analysis. If user wants to use isolated cells for culturing purpose, one must work under sterile conditions.

The kit is divided in two parts:

1. Mononuclear cells (MNC) isolator
2. Isolation of specific Cells with magnetic beads

Equipment needed:

1. Magnet rack
2. Pipettor and Pipettetips
3. Laminar flow (Optional; if user has to work under sterile conditions)
4. Washing as well as culturing media solutions e.g. PBS
5. Centrifuge (if user wants to isolate the MNC with this kit)
6. Microscope (if user wants to view the cells)

Contents of the kits:

- Tube A MNC isolator
- Tube B Magnetic beads
- Tube C Washing solution
- Tube D Blocking solution

A. Isolation of Mononuclear cells: Mononuclear cells must be isolated with Genekam Quick MNC isolation as it will give huge number of cells from small volume of blood like 1 ml or less. One can also use other MNC isolation methods like density gradient method but the number of MNC with such method will be less than Genekam Quick MNC isolation. If the user has isolated them with its own method, please go to step B magnetic beads isolation and ignore it.

1. MNC Isolator (Tube A) should be warmed at 37⁰C by keeping it in Incubator / other heating instrument for 10 minutes. Add blood and MNC isolator in ratio of 1:5 i.e. 1 ml blood and 4 ml MNC isolator in a tube and mix it (volume of blood for isolation can be increased or decreased as per demand).

Hint: If user does not have the possibility to warm it. Please proceed without it as you may have some erythrocytes in your pellet!

GENEKAM T-CELL (CD4 POSITIVE) ISOLATION KIT

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2. Keep at room temperature for 7-10 minutes with occasional shaking.
3. Now centrifuge this mix at 1650 rpm for 7 minutes. Discard the supernatant carefully without losing the pellet at the bottom. Wash the pellet twice with washing solution (Tube C) and centrifuge it at 1650 rpm for 7 Minutes while keeping the pellet intact.

At the end user has pellet. Add 100 ul of washing solution (Tube C) to dilute the pellet and calculate the number of cells with trypan blue. (If user does not have this, please ignore and proceed ahead). But it is better to calculate the number of cells to have better results.

There may be some erythrocytes in the pellet, but they will not interfere during magnetic bead isolation process. Now proceed to magnetic beads isolation.

B. Isolation with magnetic beads:

1. Add 5 ul blocking solution (Tube D) on the pellet and keep at room temperature for 10 minutes.
2. Mix the cells with washing solution in such a way that one has 5×10^6 or 10^7 cells per 100ul. (If user does not calculate the number of cells, please proceed directly to next step directly. Hint: dilute the cells in washing solution roughly e.g. 300 ul so that they can suspend in solutions, if user has too many cells in pellet!) If the number of cells are less 5×10^6 , please add them in 100 ul washing solution, user will get isolated CD4 cells from this volume).
3. Add 10 ul of magnetic beads (Tube B) in diluted cells in washing media (Tube C). Keep the cells with magnetic beads for 20-30 minutes at 4°C with occasionally shaking. Use 10 ul magnetic beads per 10^7 cells.
4. Add 1 ml washing buffer (Tube C) to the tube. Put the tube in magnetic rack for 2-3 minutes and user will see that the beads are attracted towards to one side. Please remove the fluid carefully with the pipettor without disturbing magnetic beads on the wall (fluid contains other cells than the targeted cells If user needs them for other purpose, they can be collected and stored!). Wash the beads in 1ml or 2ml washing solution (Tube C) and put the tube in magnetic rack for 2 minutes, where the magnetic beads will be attracted towards wall and remove the supernatant without disturbing the magnetic beads. Now user can collect magnetic beads in 100 ul (or less) in washing solution or in PBS. The targeted cells are attached to magnetic beads. (Hint: one can view isolated cells under microscope. Take 1 ul on the slide and view them under microscope). These cells can be used to isolate the RNA/DNA, to do the cell culturing as well as microscopic analysis etc. Please note that beads are nontoxic to cells, hence they will not interfere during the RNA / DNA isolation process. Genekam has used them for different analysis in its laboratory.

Tips: magnetic beads can be removed from the cells with methods, which cleave the antibodies at specific bonds. Please ask for these methods.

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If you should find any mistakes, please let us know. Thank you.

Suggestion:

This manual has been written specifically for beginners, hence persons with experience in PCR must use their experience to keep each step as small as possible e.g. you should calculate the amount of the needed chemicals, before starting with testing.

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