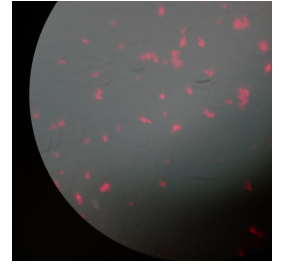




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A simple and fast single step method to isolate and stain the CD27 B cells (plasma cells) with quantum dots magnetic beads antibody conjugate for fluorescent microscopy



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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

Background Today, mononuclear cells are stained in a two-step process: in the first step, the cells are isolated, and in the second step, they are stained. This two-step process takes a long time before the cells can be observed under a fluorescence microscope. In addition, quantum dots have further advantages over conventional dyes such as FITC, Cy3, Cy5, etc., as they do not fade and emit fluorescence in a narrow range. We decided to develop a new method in which the cells can be isolated and stained in a single step using quantum dot magnetic bead antibody conjugates for CD27-positive B cells.

Material and Methods CD27 cells from mononuclear cell cultures were stained and isolated simultaneously in magnetic racks using quantum dot magnetic bead antibody conjugates with two different wavelengths (510 and 700 nm). They were then observed under a fluorescence microscope.

Results CD27 cells were successfully isolated and stained within 30 minutes, emitting a specific fluorescence under the microscope so that only the specific CD27 B cells were observed and no other cells were present. Under the normal microscope, the magnetic beads adhered to their surfaces.

Conclusions This may be the first report of the successful development of a rapid one-step method for isolating and staining CD27 B cells from mononuclear cells using quantum dot magnetic bead antibody conjugates within 30 minutes, compared to the two-step method, which takes more than an hour. This method opens up new possibilities for immunotherapies and clinical applications.

Image Attribution

Quantum dot magnetic bead conjugate Genekam

Materials

Quantum dots magnetic beads antibody conjugate (MICROBOSS Nanomedicine GmbH, Germany)

Magnetic racks (Genekam Biotechnology AG, Germany)

PBS (Washing buffer, Lonza, USA)

Fluorescent microscope (Zeiss, Germany)

50 ml sterile tubes

Microscopic slides (Thermofischer, Germany)

5 ml tubes



Safety warnings



1. Please use a high-quality fluorescence microscope, as inferior and cheap microscopes do not deliver results and are therefore a waste of time.
2. Please keep away your mobile telephone and camera from the magnetic rack as the magnet can damage your display.
3. Work under laminar flow.

Ethics statement

Since human blood samples are being used, approval from an ethics committee may therefore be required. Please check this point.

Before start

Please read the protocol properly before start of the experiment.



Protocol A: There are two protocols: A and B. Use one protocol.

- 1 Centrifuge 5 ml of cell culture of human mononuclear cells at 18000 rpm and wash twice with PBS. Collect the pellet.
- 2 Add 2 μ l of quantum dot magnetic bead antibody conjugates (510 nm and 700 nm) on the pellet and incubate it in the dark for 10 minutes with intermittent mixing.
- 3 Wash the cells with 5 ml PBS while placing on a magnetic rack for 3 minutes. Remove the fluid while keeping pellet in the tube.
- 4 Suspend the pellet in 100 μ l PBS; apply 1 μ l to a slide. Let it dry and fix in ethanol for 5 minutes, and mount it the slide.
- 5 Observe the CD27 positive cells using a Zeiss fluorescence microscope with UV, blue, and green filters at various magnifications, with and without oil immersion. Attempts using a lower-cost Biobase microscope were unsuccessful. Do not waste your time using low quality microscope.

Alternative Protocol B

- 6 Inocubate the 5 ml of cell culture with 2 μ l of quantum dot magnetic bead antibody conjugate for 10 minutes.
- 7 Suspend the cells in 5 ml on a magnetic rack, discard the supernatant and wash pellet twice with 5 ml PBS.
- 8 Apply 2 μ l of the pellet after suspending it in 100 μ l PBS to a slide. Let it dry, fix it, and mount it.
- 9 Observe the CD27 positive cells under a Zeiss fluorescence microscope with UV, blue, and green filters at various magnifications, with and without oil immersion. Attempts using a lower-cost Biobase microscope were unsuccessful.

Protocol references

Bhatia, S. (2025). A Simple and Fast Single-Step Method to Isolate and Stain CD27 B Cells (Plasma Cells) Using Quantum Dot Magnetic Bead Antibody Conjugates for Fluorescent Microscopy. *Medical Science and Discovery*, 12(9), 284–287. <https://doi.org/10.36472/msd.v12i9.1315>

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