

# Stem cell Isolation

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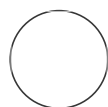
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Isolation of rest of the trypsinized stem cells from empty cell culture flasks with CD90 specific magnetic beads

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We use this protocol and it's working

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## ABSTRACT

In all laboratories working with the stem cells, it is common practice to isolate these cells with help of trypsin solution because these cells are grown on the plastic surface of cell culture flasks as well as plates. The stem cells like mesenchymal stromal stem cells (MSC) adhere to the plastic surface, which is one of their important properties. After trypsin solution treatment, the cells can be detached from their plastic surface and go into the solution. The user centrifuges such solution to get the pellet of these cells. After that the plastic flasks are observed under the microscope to find whether all cells are detached from the surface fully. Once there are no attached cells, the flasks are thrown away as waste. In our laboratory, we found if PBS is added to these empty flasks, there are a lot of stem cells still inside. The stem cells grow slowly and have limited number of growth cycles. Most of the times, user needs a huge number of cells for the performance of different applications. Such stem cells may be a limiting factor to conduct these experiments. We used CD90 magnetic beads to isolate the rest of trypsinized stem cells swimming in the PBS in such empty flasks and used such cells to grow them again in the new cell culture flasks as well as plates along with using them to conduct other experiments.

Principle of this method is to use MSC specific marker magnetic beads, which will attach on the surface of MSC. They are pulled out with the magnetic field in a magnetic rack. CD90 is one of important markers of MSC.

## IMAGE ATTRIBUTION

Stem cell picture

## MATERIALS

Materials needed:

Mesenchymal stromal stem cells, trypsin solution (Biochrome), magnetic rack for 15 ml tubes (Genekam), CD90 magnetic beads (MICROBOSS Nanomedicine GmbH), sterile PBS, Dulbecco's Modified Eagle Medium (DMEM; Biochrome), sterile 15 ml tubes, cell culture plates or flasks, centrifuge, pipettor and sterile Pipette tips, MSC cell culture media and CO<sub>2</sub> incubator.

## SAFETY WARNINGS



Warning 1. Magnetic beads with isolated cells can be suspended in the other media like PBS to isolate of nucleic acid or conduct the flow cytometry assays etc. User must work in sterile laminar flow, If the isolated cells are to be cultured for further use.

2. Please do not throw away the magnetic beads during isolation step as stem cells are attached to these beads.

3. User can use its own culture media to grow MSC.

## BEFORE START INSTRUCTIONS

Please read the material list and protocol carefully.

- 1 Grow the stem cells in the flask in cell culture media till they reach 80% confluency.
- 2 Remove the cell culture media.
- 3 Add 15 ml PBS to wash the cells and remove the solution. This is washing step, which is optional.
- 4 Add trypsin solution to this. Usually it is 6 – 10 ml depending on the manufacturer.

- 5 Keep it for 20-30 minutes in CO<sub>2</sub> incubator at 37 °C.
- 6 Now scrap off the cells with pipette tip or gentle shaking and collect the fluid containing cells. Repeat this step till the user does not see any cells attached on the surface of the trypsinized flask under the microscope. This fluid can be centrifuged to get the pellet of cells for further use.
- 7 Now add the 10 ml PBS to this trypsinized flask and collect this fluid in 15 ml tube.
- 8 Add 50 µl of CD90 magnetic beads in 15 ml tube. Wait for 30 minutes.
- 9 Put this 15 ml tube in magnetic rack for minutes. User is going to observe that the magnetic beads will be pulled towards the magnet in the rack.
- 10 Remove the fluid while titling magnetic rack slowly or one can use pipettor to remove the fluid gently, while keeping the magnetic beads in the tube.  
  
Hint: Do not throw away the magnetic beads as magnetic beads must remain in the tube because this is very important!
- 11  
  
Add 5 ml DMEM to suspend the magnetic beads in it. Take 1 µl of the beads on the microscopic slide and observe it under microscope to see isolated stem cells. On their surfaces, user will observe that the magnetic beads are attached. Now user has isolated the stem cells, which can be cultured in cell culture plates or flasks in cell media. The beads remain on the surface of cells. Please see the pictures in the publication in this protocol. These isolated cells can be used to conduct other experiments like molecular analysis or flow cytometry experiments.

