Isolation of Monocytes from Whole Blood With Magnetic Negative Sorting

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Abstract

Citation: Brandt Pence Isolation of Monocytes from Whole Blood With Magnetic Negative Sorting. **protocols.io** dx.doi.org/10.17504/protocols.io.kwtcxen
Published: 21 Nov 2017

Materials

EasySep Direct Human Monocyte Isolation Kit 19669 by Stem Cell Technologies
EasyEights Magnet 18103 by Stem Cell Technologies
K2EDTA Vacutainer Tubes 366643 by Bd
14 ml round-bottom tubes 352057 by Corning
PBS without Ca2 or Mg2 10010-031 by Gibco, ThermoFisher
✓ 0.5M EDTA AM92606 by Contributed by users
✓ Pipettes and P1000, P200 tips by Contributed by users

Protocol

Step 1.

Materials to Prepare:

Isolation Buffer: 1 mM EDTA in phosphate-buffered saline (PBS):

-Add 2 ml 0.5 M EDTA to a 1 L bottle of sterile PBS.

-Maintain sterility.

-Store at room temperature.

-Usable at least 6 months.

Media

-Various media can be used, depending on downstream assay.

-For counting using a Scepter cell counter (Millipore), serum-free medium is necessary.

Step 2.

Collect blood into K2EDTA vacutainer tube and invert several times. Store at RT until use.

Step 3.
Aliquot whole blood into 14 ml tubes. Each tube receives 4 ml of blood. A successful isolation will yield 1-2×10^6 total cells (>90% monocytes) per 4 ml of blood.

Step 4.
Vortex RapidSpheres (brown reagent) from monocyte isolation kit well (15-30 sec). Vortex isolation cocktail (clear reagent) from monocyte isolation kit (5 sec).

Step 5.
Add 200 µl RapidSpheres and 200 µl isolation cocktail to each 14 ml tube. Pulse vortex tubes 5-10 times to mix. Incubate 5 min at room temperature.

Step 6.
Add 8 ml isolation buffer to each tube and invert 5 times to mix (make sure to hold tube cap down tightly to avoid spilling sample). Move tubes to magnet and incubate 5 min at room temperature.

Step 7.
Remove supernatant from tubes by pipetting and transfer to new 14 ml tube. Supernatants will be a somewhat clarified red color. Red blood cells will not stick well to the magnet during the first transfer step (this will not affect performance). For best yield, transfer 0.5 ml of red blood cells from as well.

Step 8.
Add 200 µl RapidSpheres and 200 µl isolation cocktail to each 14 ml tube. Invert 5 times to mix (make sure to hold tube cap down tightly to avoid spilling sample). Incubate 5 min at room temperature.

Step 9.
Move tubes to magnet and incubate at least 5 minutes at room temperature. A 10 minute incubation on this step appears to improve red blood cell removal and does not affect cell viability.

Step 10.
Remove supernatant from tubes by pipetting and transfer to new 14 ml tube. Supernatants will now be a clarified yellow color similar to blood plasma. Red blood cells should stick well to the magnet during this transfer step. Do not remove or disturb red blood cells.

Step 11.
Move new tubes directly to the magnet and incubate 5 minutes at room temperature.

Step 12.
Remove supernatant from tubes by pipetting and transfer to appropriate tubes for centrifugation (note 1). Supernatants will be a clarified yellow color similar to blood plasma. Residual red blood cells should form a very thin line on the back of the tube against the magnet. Do not remove or disturb red blood cells.

NOTES
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(1) We use 5 ml tubes (3 per 14 ml tube) for this step as we do not have a good way to centrifuge the 14 ml tubes in our lab. Choice of tube will depend on availability of rotors for the centrifuge.

Step 13.
Centrifuge cells at 500 × g for 5 min in refrigerated centrifuge (4-8degC) to pellet cells.

Step 14.
Aspirate supernatants and resuspend in prepared media or buffer for counting (note 2). A volume of 75-100 µl of media per 1 ml of initial blood that was processed seems to work well and consistently gives a final cell concentration of 4-6 × 10^6 cells / ml.

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(2) If red blood cells are visible in the pellet, a red blood cell lysis step can be inserted here. This is usually not needed and will somewhat decrease total cell yield.

Step 15.
Count cells using routine methods (note 3). Cell viability should be near 100%.

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(3) We use a Scepter counter with a 40 µm sensor and dilute the cell suspension 10× in media prior to counting (20 µl cells in 180 µl media). Note that the Scepter requires serum-free medium as presence of serum in the media will clog the sensor.

Step 16.
Proceed to downstream applications. Cell purity can be checked using flow cytometer with an anti-CD14 antibody (note 4).

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(4) Note that this particular kit contains anti-CD16 antibodies and thus depletes intermediate ($CD14^{+}CD16^{+}$) and non-classical monocytes ($CD14^{dim}CD16^{++}$). A monocyte kit without CD16 depletion (#19058) is available from Stem Cell Technologies.