Isolation and culture of macrophages from human peripheral blood

Reagents
- Human buffy coat, not older than 8 hours.
- Tissue culture dishes for differentiation.
- Histopaque-1077.
- Culture medium: medium used for human T lymphocytes: RPMI 1640 + 50 ml FBS, 1 bottle of streptomycin/penicillin/glutamine, 5 ml Na-pyruvate, 5 ml non-essential amino acids, 175 μl β-mercaptoethanol from the stock in the fridge), 10 ml HEPES (1M stock). The medium must stored in the fridge and be discarded after 10-15 days or if it changes color.
- Differentiation medium: culture medium + 5% FBS + 50 ng/ml rHu M-CFS. Prepare the whole medium but only add the M-CSF and FBS to the amount of medium to be used for the day.
- PBS (without Ca²⁺ and Mg²⁺) + 2% FBS.
- PBS with Ca²⁺ and Mg²⁺.

Methods
Find out the volume of buffy coat from the documentation, it is usually around 60 ml.
Prepare 50 ml conical tubes with 15 ml Histopaque-1077 in each. You will need one tube per 10 ml buffy coat. The Histopaque must be used at room temperature.
Wipe a pair of surgical scissors and the tubing of the buffy coat pouch with 70% ethanol.
Cut the end of the tubing and pour the buffy coat into a 75 cm² tissue culture flask.
Dilute the buffy coat with the same volume of PBS + 2% FBS and mix gently.
Layer the diluted buffy coat onto the Histopaque very carefully to avoid mixing the two.
**Immediately** centrifuge for 20 minutes at room temperature at 1,200 g, with the accelerator and the brake off (Histopaque is toxic to cells so do not let the cells sit on the gradient).
After centrifugation, **immediately** collect the ring of cells at the interface between the Histopaque and the diluted plasma using a Pasteur pipet and a latex bulb. Collect in a 50 ml conical tube.
**Immediately** fill the tube up to 50 ml with PBS + 2% FBS and centrifuge to pellet the cells (250 g at room temperature for 10 minutes, with accelerator and brake on).
**Immediately** discard the supernatant, break the pellet by taping the tube, and resuspend the cells in 50 ml PBS + 2% FBS.
Take a sample for counting.
Centrifuge to pellet the cells (250 g at room temperature for 10 minutes, with accelerator and brake on).
During the centrifugation, count the cells.
Do not let the cells wait in a pellet. Discard the supernatant, break the pellet by taping the tube, and resuspend the cells at 1-5 million per ml of culture medium (serum-free).
Plate into 10 cm culture dishes (10 ml/dish).
Incubate for 1-2 hours at 37°C, 5% CO₂.
Look at the plated cells under the microscope to check you have adherent cells.
Hold the dish at an angle and remove the supernatant.
Add warm PBS (with Ca²⁺ and Mg²⁺); 5 ml per culture dish) at the bottom of the plate (avoid making it run along the adherent cells).
Swirl gently.
Discard the supernatant.
Repeat the last steps (adding PBS, swirling, and collecting).
Add 10 ml differentiation medium to the plates.
Let the cells differentiate for 7 days, replacing the differentiation medium every 2-3 days, and checking the cells daily.