PARSE BIOSCIENCES

PARSE DEVELOPED PROTOCOL

Isolating and Purifying PBMCs from Whole Blood

For processing whole blood at Parse Biosciences, we recommend:

- EasySep™ Direct Human PBMC Isolation Kit, Stemcell Technologies, Catalog # 19654 EasySep™ Direct Human PBMC Isolation Kit
- We use this with the magnet “The Big Easy” also from Stemcell Technologies, catalog #18001 “The Big Easy" EasySep™ Magnet

The SOP that Parse Biosciences recommends:

- EasySep™ Direct Human PBMC Isolation from Whole Blood.
- From the EasySep™ Direct Human PBMC Isolation from Whole Blood SOP on page 2, “The Big Easy” column (seen below for reference)

Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Tables 2 - 4 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 2. EasySep™ Direct Human PBMC Isolation Kit Protocol for WHOLE BLOOD

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	EasySep™ (Catalog #18000)	"The Big Easy" (Catalog #18001)
1	Prepare sample within the volume range.	1 - 2 mL	1 - 6 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add EDTA to sample.	At a final concentration of 6 mM EDTA	At a final concentration of 6 mM EDTA
3	Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
4	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to double the original sample volume	Top up to double the original sample volume
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
6	Add RapidSpheres™ to sample and mix.	50 µL/mL of original sample volume NOTE: No incubation, IMMEDIATELY proceed to next step	50 µL/mL of original sample volume NOTE: No incubation, IMMEDIATELY proceed to next step
7	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
8	Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring the enriched cell suspension* into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
9	Add RapidSpheres™ to the new tube containing the enriched cells and mix.	Use same volume as in step 6 NOTE: No incubation, IMMEDIATELY proceed to next step	Use same volume as in step 6 NOTE: No incubation, IMMEDIATELY proceed to next step
10	Remove the tube from the magnet; place the tube from step 9 (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes
11	Pick up the magnet, and in one continuous motion invert the magnet and tube,† pouring the enriched cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
12	Remove the tube from the magnet; place the tube from step 11 (without lid) into the magnet and incubate for a third separation.	RT for 5 minutes	RT for 5 minutes
13	Pick up the magnet, and in one continuous motion invert the magnet and tube,† pouring the enriched cell suspension into a new tube.	Isolated cells are ready for use	Isolated cells are ready for use

RT - room temperature (15 - 25°C)
* Following the first magnetic separation, the collected cells may contain a significant amount of RBCs and may look similar to the original unprocessed human whole blood sample.
† To minimize RBC contamination in the isolated cells, pour off the sample along a clean area of the tube (i.e. the opposite side to where the sample was poured in).

Please note steps that are not explicitly in the protocol:

1. Make buffer media with final concentrations of: 1X PBS containing 2% FBS and 1 mM EDTA
2. Add 2 mL of whole blood to the specified tube (14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g., Catalog #38008))
3. Add EDTA to the sample to a concentration of 6 mM EDTA
4. *Follow EasySep™ protocol steps 3-13 as written.* If you started with 2 mL of blood, then there should be ~ 4 mL of purified enriched cell solution.
5. Spin enriched solution 200xg for 10 mins at 4°C.
6. Resuspend in 100 µL Cell Prefixation Master Mix and dilute 1:2 to count cells

7. Move up to 1 million cells into the Cell Fixation process and top up to the correct volume of Cell Prefixation Master Mix using the current version of the Cell Fixation User Guide.

Critical: For processing PBMCs from whole blood, samples may be processed up to 48 hours post-draw. At the time of the blood draw, it is strongly recommended to immediately place and retain samples at 4°C until they are processed for fixation in order to maintain their biological transcriptional state. However, samples should be processed at room temperature for the magnet steps detailed in the EasySep™ protocol.

Note: Please check the Parse Support Suite for the most up-to-date User Guides.

For processing Ficoll-Paque PBMCs (with trace amounts of RBCs), we recommend:

- EasySep™ RBC Depletion Reagent, from Stemcell Technologies, Catalog # 18170 EasySep™ RBC Depletion Reagent

- We use this with the magnet “The Big Easy” also from Stemcell Technologies, catalog #18001 The Big Easy" EasySep™ Magnet

The SOP that Parse Biosciences recommends:

- EasySep™ RBC Depletion
- Steps that are not explicitly in the protocol but should be performed:
    1. Aliquot 1-5 mL of Ficoll-Paque PBMC media to a 15 mL tube
    2. Add EDTA to a total concentration of 6 mM EDTA in media and follow the EasySep™ protocol as written. If you started with 2 mL of sample, then there should be ~ 4 mL of purified enriched cell solution.
    3. Spin enriched cell solution at 200xg for 10 mins at 4°C, resuspend in 100 µL of Cell Prefixation Master Mix, and dilute 1:2 to count cells
    4. Finally, move up to 1 million cells into the Cell Fixation process and top up to the correct volume of Cell Prefixation Master Mix using the current version of the Cell Fixation User Guide.

Note: We DO NOT recommend using RBC lysis for processing whole blood. RBC lysis can damage the cell membrane of PBMCs and lead to poor scRNA-seq data quality.