Turkish Journal of Immunology, 14(1)

Comparative Evaluation of Magnetic Cell Separation Systems Based on Cell Recovery and CD14 mRNA Enrichment

Büyüker A, Sönmez HE, Sözeri B, Karadenizli A. Comparative evaluation of magnetic cell separation systems based on cell recovery and CD14 mRNA enrichment. Turk J Immunol. 2026;14(1):45–58

DOI: 10.36519/TJI.2026.1040

The authors provide the tables and figures in the supplementary appendix to give readers detailed information about the study.

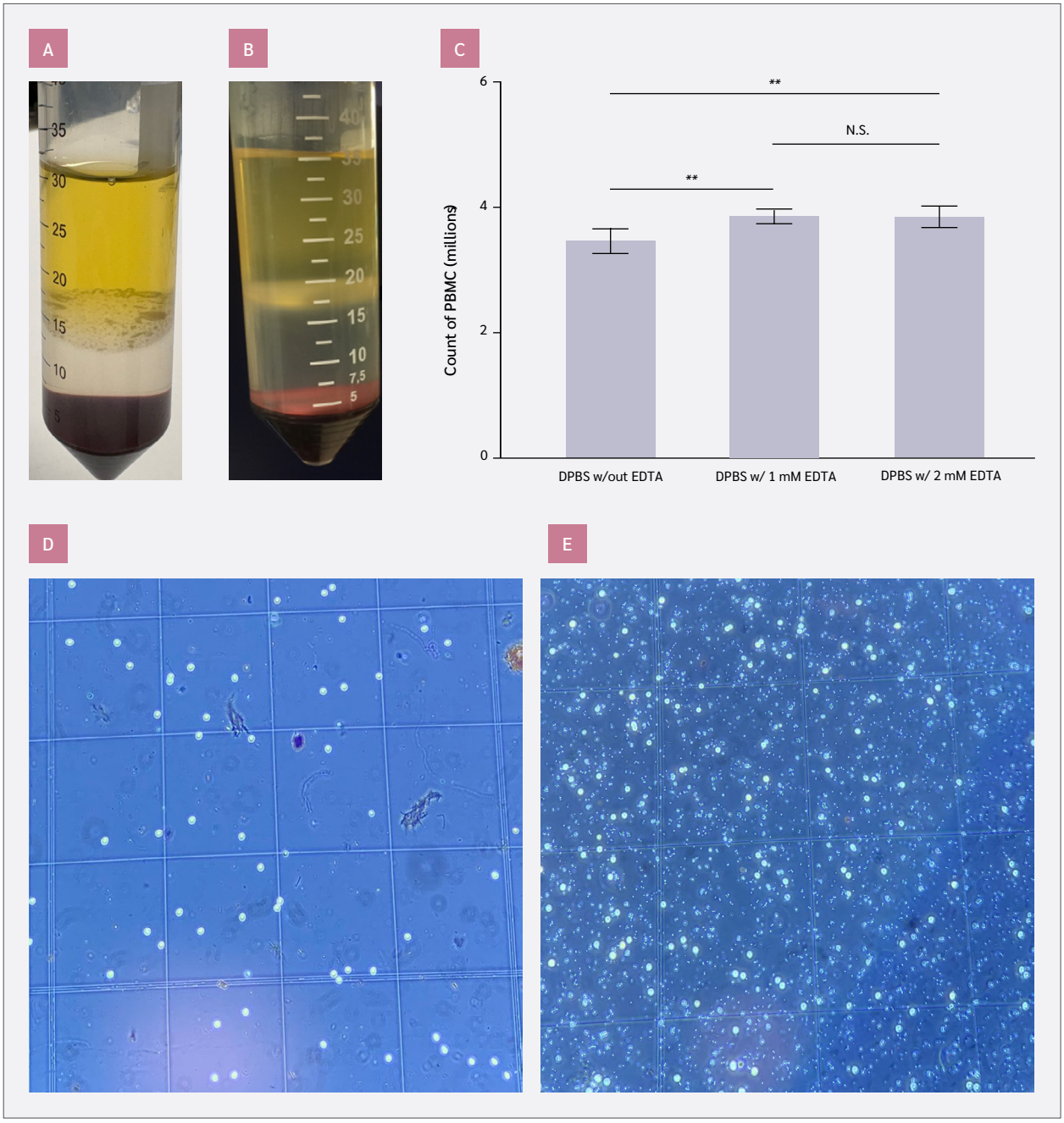

Figure S1. Optimization of the working conditions for PBMC isolation.

Representative images of the buffy coat after density gradient centrifugation showing **(A)** cell clumping and **(B)** a homogeneous PBMC layer. **(C)** PBMC counts obtained during the isolation process in the presence of 1 mM ethylenediaminetetraacetic acid (EDTA), 2 mM EDTA, or in the absence of EDTA. Representative microscopic images obtained during trypan blue–based cell counting of sorted fractions following **(D)** column-based and **(E)** column-free separation. p values were 0.0015 for Dulbecco phosphate-buffered saline (DPBS) without EDTA vs DPBS with 1 mM EDTA, 0.0044 for DPBS without EDTA vs DPBS with 2 mM EDTA, and 0.8376 for DPBS with 1 mM EDTA vs DPBS with 2 mM EDTA.

NS: Nonsignificant.

Table S1. Demographic and clinical characteristics of healthy blood donors (n=8).

Sample ID	Sex	Age (years)	CRP (mg/dL)	ESR (mm/h)	BMI (kg/m²)
#1	Male	10	0.67	8	17.7
#2	Female	8	0.81	10	16.6
#3	Male	12	0.48	12	20.1
#4	Female	8	0.72	10	16.8
#5	Female	9	0.62	10	18.3
#6	Female	11	0.46	12	18.9
#7	Male	11	0.56	8	19.2
#8	Male	14	0.68	14	21.0
Mean ± SD		10.38 ± 2.07	0.63 ± 0.12	10.5 ± 2.07	18.58 ± 1.54

BMI: Body mass index, **CRP:** C-reactive protein, **ESR:** Erythrocyte sedimentation rate.

Table S2. Troubleshooting guide for PBMC isolation and magnetic cell separation procedures.

Problem	Possible cause	Solution
Separation failure in PBMC isolation (Mixed layers or diffuse buffy coat)	Blood > 6 h old or cold environment (+2–8°C)	Process fresh blood as soon as possible
	Disturbing the ficoll while blood: DPBS mixture layering	Dilute whole blood with DPBS, w/out Ca^{++} and Mg^{++}, or analog buffer prepared 1x
	Centrifuge brake ON	Set brake OFF, centrifuge 400–500 × g for 20–40 min at RT
	Incorrect blood: DPBS mixture ratio	Layer the mixture slowly down tube wall
Low PBMC yield / Poor viability	Poor buffy coat collection	Aspirate entire buffy coat, maybe take the upper plasma layer first Dilute blood 1:1 with PBS + 2% FBS before separating
	Clumpy buffy coat layer	Do not keep the blood and reagents at cold (+2–8°C) Perform PBMC isolation steps (especially centrifugation) at RT (18–22°C)
	Red blood cell (RBC) contamination	Add RBC lysis before isolation
	Anticoagulant blood	Use EDTA tubes rather than other types
Low cell recovery in the flow-through fraction	Insufficient antibody labeling	• Optimize bead-to-cell ratio (double the reagent volumes if cells more than 10^7) • Perform antibody labeling on ice/4°C to reduce non-specific interactions • Use a gentle shaker/rotator to ensure uniform antibody distribution across all cell surfaces
	Non-specific binding	Use Fc receptor block solution during or prior to antibody labelling
	Clumpy cells	Increase BSA up to 2% and use freshly prepared EDTA in MACS buffer to reduce clumping
	Clogging the column by aggregates	• Count the cells carefully, preferably by an independent (blinded) evaluator. • Do not exceed the column capacity (e.g., LS ≤ 10^8/column) • Filter samples through a 30 or 40 µm strainer before separation • Add DNAse I to eliminate free DNA from broken cells that leads to aggregation • Use DPBS w/out Ca^{++} and Mg^{++}, since Ca^{++} and Mg^{++} in buffer promote adhesion • Keep cells gently rotating during antibody binding
Low DNA/RNA purity		Wash the magnetically selected cells with DPBS w/out Ca^{++} and Mg^{++} to remove MACS buffer components

Table S3. The sequences of oligonucleotide primers.

Gene	Primer sequences (5'-3')
CD14	F: AAGACTTATCGACCATGGAGC R: GCATGGATCTCCACCTCTAC
CSF1R	F: ATCCGGCTGAAAGTGCAGAA R: TGTTGAGGGATTGCGAGCTT
CD68	F: GGGGCATCTCTGTACTGAACC R: AATGTCCACTGTGCTGCGTG
GAPDH	F: GCACCGTCAAGGCTGAGAAC R: TGGTGAAGACGCCAGTGGA

F: Forward, **R:** Reverse.