Biocoll

Biocoll separating solution does contain a polymer with a molecular weight of approx. 400000 Dalton. Densities of up to 1.1 g/ml can be adjusted using this hydrophilic polymer. For optimal pH and osmolality, adjusting Biocoll with an acid, preferably amidotrizoeic acid (ATA), and sodium hydroxide is required. Biocoll with densities of 1.077 g/ml (cat. no. L 6113/5) and 1.090 g/ml (cat. no. L 6125) are already adjusted accordingly.

Product	Cat. No.	Unit
Biocoll density 1.077 g/ml, isotone Storage temperature: RT (room temperature)	L 6113 L 6115	100 ml 500 ml
Biocoll with 10 mM HEPES density 1.077 g/ml, isotone Storage temperature: RT	L 6713 L 6715	100 ml 500 ml
Biocoll, density 1.09 g/ml isotone Storage temperature: RT	L 6125	500 ml
Biocoll, density 1.10 g/ml isotone Storage temperature: RT	L 6155	500 ml

Recommendations:

The ready-to-use separating solution Easycoll is already adjusted to physiological conditions of osmolality and pH value. But you can also adjust a required density yourself diluting Easycoll correspondingly.

Tab. 36: Instruction how to dilute the stock solution of Biocoll (density 1.090 g/ml; cat. no. L 6125) with PBS (cat. no. L 1825/0)
All amounts given for a temperature of +20 °C. Only PBS w/o Ca^{2+}, Mg^{2+} is suitable, since these ions will influence agglomeration and increase formation of cell clusters.

Desired density (g/ml)	Vol. % of Biocoll (density 1.090 g/ml)	Vol. % of PBS
1.080	88.2	11.8
1.075	82.4	17.6
1.070	76.5	23.5
1.065	70.6	29.4
1.060	64.7	35.3
1.055	58.8	41.2
1.050	52.9	47.1
1.045	47.1	52.9
1.040	41.2	58.8
1.035	35.3	64.7
1.030	29.4	70.6

Separating lymphocytes using Biocoll

1. Biocoll separating solution (D=1.077 at +20 °C) is given to 15 or 25 ml centrifugal tubes in volumes of 7 ml and 10 ml, resp.
2. Equal parts of heparinized whole blood (50 U/ml heparin, stabilizer-free) and culture medium are mixed and carefully applied over Biocoll separating solution.
3. Centrifuge 1200xg for 20 min.
4. Take layer of enriched (70 – 100 %) lymphocytes (between plasma and Biocoll) with a Pasteur's pipette and wash twice in culture medium:
   - for 10 min at 300xg
   - for 10 min at 200xg
5. Cell count as usual.