Eosinophil Isolation from Human Blood

1. One part 4.5% Dextran in PBS was added to 5 parts peripheral blood collected from donors.
2. Leukocyte-rich plasma was mixed 1:1 with PBS + 2 mM EDTA and then applied (25 ml per gradient) to a Percoll gradient (1.5 ml 10x HBSS, 9.5 ml Percoll, 4.5 ml H2O) and spun at 1300 rpm (~500 x g) for 30 minutes.
3. Granulocytes were collected and red blood cells were lysed by hypotonic lysis.
4. Granulocytes were incubated with anti-CD16 MACS microbeads (Miltenyi Biotec) (1 µl per 1 x 106 cells) for 30 minutes at 4oC.
5. Cells were then applied to a MACS column, and eosinophils were eluted.
6. Eosinophil purity was confirmed by cytospin and DiffQuick staining and was routinely >95%, and viability was >98%, as assessed by trypan blue exclusion.
7. Eosinophils were resuspended at a density of 1 x 106 cells/ml in RPMI + 10% FBS + 1% penicillin/streptomycin and cultured at 37oC until they were used in transmigration assays or for protein isolation.