Video Article

Enrichment of NK Cells from Human Blood with the RosetteSep Kit from StemCell Technologies

Christine Beeton, George K. Chandy

Department of Physiology and Biophysics, University of California, Irvine

Correspondence to: Christine Beeton at cbeeton@uci.edu

URL: <http://www.jove.com/index/Details.stp?ID=326>

DOI: 10.3791/326

Citation: Beeton C., Chandy G.K. (2007). Enrichment of NK Cells from Human Blood with the RosetteSep Kit from StemCell Technologies. JoVE. 8. <http://www.jove.com/index/Details.stp?ID=326>, doi: 10.3791/326

Abstract

Natural killer (NK) cells are large granular cytotoxic lymphocytes that belong to the innate immune system and play major roles in fighting against cancer and infections, but are also implicated in the early stages of pregnancy and transplant rejection. These cells are present in peripheral blood, from which they can be isolated. Cells can be isolated using either positive or negative selection. For positive selection we use antibodies directed to a surface marker present only on the cells of interest whereas for negative selection we use cocktails of antibodies targeted to surface markers present on all cells but the cells of interest. This latter technique presents the advantage of leaving the cells of interest free of antibodies, thereby reducing the risk of unwanted cell activation or differentiation. In this video-protocol we demonstrate how to separate NK cells from human blood by negative selection, using the RosetteSep kit from StemCell technologies. The procedure involves obtaining human peripheral blood (under an institutional review board-approved protocol to protect the human subjects) and mixing it with a cocktail of antibodies that will bind to markers absent on NK cells, but present on all other mononuclear cells present in peripheral blood (e.g., T lymphocytes, monocytes...). The antibodies present in the cocktail are conjugated to antibodies directed to glycophorin A on erythrocytes. All unwanted cells and red blood cells will therefore be trapped in complexes. The mix of blood and antibody cocktail is then diluted, overlayed on a Histopaque gradient, and centrifuged. NK cells (>80% pure) can be collected at the interface between the Histopaque and the diluted plasma. Similar cocktails are available for enrichment of other cell populations, such as human T lymphocytes.

Protocol

A detailed protocol is included with each Rosette Sep isolation cocktail and is available online on the StemCell technologies website ¹.

Discussion

The cells isolated using this technique can be further separated into different populations using other cell separation kits.

References

1. Procedures and instruction manuals. <http://www.stemcell.com/technical/technicalrsproc.aspx>