

Video Article

Isolation of CD4+ T cells from Mouse Lymph Nodes Using Miltenyi MACS Purification

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Abstract

Isolation of cells from the primary source is a necessary step in many more complex protocols. Miltenyi offers kits to isolate cells from several organisms including humans, non-human primates, rat and, as we describe here, mice. Magnetic bead-based cell separation allows for either positive selection (or cell depletion) as well as negative selection. Here, we demonstrate negative selection of untouched or na ve CD4+ helper T cells. Using this standard protocol we typically purify cells that are = 96% pure CD4+/CD3+. This protocol is used in conjunction with the protocol Dissection and 2-Photon Imaging of Peripheral Lymph Nodes in Mice published in issue 7 of JoVE, for purification of T cells and other cell types to adoptively transfer for imaging purposes. Although we did not demonstrate FACS analysis in this protocol video, it is highly recommended to check the overall purity of isolated cells using the appropriate antibodies via FACS. In addition, we demonstrate the non-sterile method of T cell isolation. If sterile cells are needed for your particular end-user application, be sure to do all of the demonstrated procedures in the tissue culture hood under standard sterile conditions. Thank you for watching and good luck with your own experiments!

Protocol

Please read and follow the protocol included in the Miltenyi kit or online at <http://www.miltenyibiotec.com/>.

Discussion

Isolation of cells from the primary source is a necessary step in many more complex protocols. Miltenyi offers kits to isolate cells from several organisms including humans, non-human primates, rat and, as we describe here, mice. Magnetic bead-based cell separation allows for either positive selection (or cell depletion) as well as negative selection. Here, we demonstrate negative selection of untouched or na ve CD4+ helper T cells. Using this standard protocol we typically purify cells that are = 96% pure CD4+/CD3+. Although we did not demonstrate FACS analysis in this protocol video, it is highly recommended to check the overall purity of isolated cells using the appropriate antibodies via FACS. In addition, we demonstrate the non-sterile method of T cell isolation. If sterile cells are needed for your particular end-user application, be sure to do all of the demonstrated procedures in the tissue culture hood under standard sterile conditions.

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References

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