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Isolation of Mononuclear Cells from Whole Blood by Density Gradient Centrifugation [↗](#)

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Working

[dx.doi.org/10.17504/protocols.io.4v3gw8n](https://doi.org/10.17504/protocols.io.4v3gw8n)**STEMCELL Technologies**Tech. support email: techsupport@stemcell.com

ABSTRACT

This protocol describes how to isolate mononuclear cells from whole blood using density gradient centrifugation. See how to layer blood over the density medium and learn two different ways of harvesting cells from the blood plasma-density medium interface.

EXTERNAL LINK

https://www.stemcell.com/how-to-isolate-mononuclear-cells-from-whole-blood-by-density-gradient-centrifugation.html?utm_source=protocolsio&utm_medium=referral

STEPS MATERIALS

NAME ▾[Lymphoprep™](#)**CATALOG #** ▾

07801

VENDOR ▾[Stemcell Technologies](#)

BEFORE STARTING


Ensure all reagents are at room temperature.

The following video demonstrates how to isolate peripheral blood mononuclear cells from whole blood using density gradient centrifugation.

- 1 Dilute the blood sample at a 1:1 volume ratio with the appropriate medium.
- 2 Add a volume of density gradient medium to a fresh tube according to the specifications for that density gradient medium.



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- 3 Gently layer the diluted blood on top of the density gradient medium. Take care not to mix the two layers.
- 4 Centrifuge  **400 x g for 30 minutes with the brake off.**
- 5 Carefully harvest the cells by inserting the pipette directly through the upper plasma layer to the mononuclear cells at the interface.



Alternatively: You can first remove the upper layer and then collect the cells.

- 6 Finally, wash the harvested cells twice in the appropriate buffer. The cells are now ready for [EasySep™ cell separations](#) or other downstream applications.



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