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## User's Guide

## Human Cord Blood CD34<sup>+</sup> Enriched Hematopoietic Stem Cells

The following describes the preferred methods for the thawing and subsequent handling of human cord blood CD34<sup>+</sup> enriched hematopoietic stem cells

*Note*: Keep cells and reagents at room temperature after the first step.

- 1. Incubate cryovials in 37°C water bath with gentle agitation until ice is nearly melted.
- 2. Immediately transfer cells to 15 ml conical tube.
- 3. Add volume of Buffer equal to ½ of original cell suspension volume in small aliquots over 5 min. Gently mix cell suspension after each small aliquot addition. [Example: for 1.0 ml cell suspension, add 0.5 ml buffer in 5 x 0.1 ml aliquots over 5 min]
- 3. Incubate cells at room temperature for 5 min.
- 4. Repeat steps 3 and 4 above.
- 5. Bring total cell suspension volume to 10 ml with Buffer (using bulk addition).
- 6. Centrifuge 10 min. at 200g to 300g.
- 7. Remove supernatant fluid, being careful to leave cell pellet undisturbed.
- 8. Add media of choice and gently suspend cell pellet with sterile pipette.

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