echnical Resources

FAQS

STEMSEP™ PROCEDURE

Q. WHAT CONCENTRATION SHOULD CELLS BE SUSPENDED AT PRIOR TO SEPARATION?

A. A cell concentration of 2-8 x 107 is acceptable. If using 1.0" columns with human cells, the cell concentration is increased to 108 cells/mL.

Q. WHAT IS THE MINIMUM SAMPLE SIZE THAT CAN BE USED?

A. The minimum volume in which cells should be suspended is 100 µL for human cell separations and 200 µL for mouse cell separations. The volume of cell suspension determines the volume of cocktail and colloid to use. If small numbers of cells are to be separated, the suspension may be less concentrated than 2x107 cells/mL.

Q. WHAT IS THE BENEFIT OF USING A PUMP?

A. The benefit is twofold: 1) It can allow up to four simultaneous separations to be performed when used in conjunction with the blue magnet (#11060). 2) It can improve purity and recovery when larger columns are used.

Q. SHOULD MY PRIMING BUFFER CONTAIN PROTEIN?

A. The priming medium should not contain protein. Use PBS, either at room temperature or degassed, without serum or other protein to prime the column from bottom up. However, the separation medium used to wash the column prior to use will contain protein.

Q. DO BUFFERS NEED TO BE DEGASSED PRIOR TO DOING STEMSEP™?

A. No. Degassing the buffer is not necessary.

Q. WHAT IS THE FUNCTION OF EDTA IN THE BUFFER?

A. EDTA prevents cells such as monocytes from adhering to the column. The effective concentration is 1–2 mM.

Q. WHY IN THE STEMSEP™ MANUAL IS IT SUGGESTED TO AVOID DEXTRAN SEDIMENTATION?

A. Our particles are coated with dextran, so if the free dextran is not washed away completely there could be some interference with colloid binding. HetaSep sedimentation is a good alternative to dextran. Please see the HetaSep protocol.

Q. CAN THE STEMSEP™ COLUMNS BE REUSED?

A. Yes, we have found that the columns can be reused up to six times without a decrease in performance, see protocol for Reusing StemSep Columns.

Q. CAN THE CELLS THAT WERE RETAINED IN THE COLUMN BE REMOVED?

No. Since our columns bind labeled cells so strongly, it is difficult to remove cells from the column even after the column is removed from the magnet.

Q. WHY IS IT SUGGESTED THAT THE ANTI-CD32 ANTIBODY BE USED WHEN ISOLATING MONOCYTES?

A. Monocytes may non-specifically bind tetramers via the Fcg RIIA on their surface, and subsequently be retained on the column. To decrease this non-specific binding, cells should be incubated with an anti-CD32 blocking reagent (Catalog #14531, #14551, and #14561) prior to immunomagnetic labeling. To use, follow the instructions in the StemSep™ Operating Manual.

STEMSEP™ COMPABILITY

Q. CAN STEMSEP™ COLUMNS BE USED FOR POSITIVE SELECTION?

A. No. Our positive selection reagents can be used with commercially available positive selection columns.

Q. CAN I USE STEMSEP™ ON REAGENTS OTHER MAGNETIC CELL SEPARATION SYSTEMS?

A. Because of the extremely small size of the StemSep™ magnetic colloid (50-150 nm), we have found that StemSepTM reagents are completely compatible with any magnet of strength equal or greater than 0.5 Tesla. Many customers have reported that their results have been equivalent or better with the economical StemSep™ reagents.

Q. WHICH SPECIES CAN STEMSEP™ BE USED WITH?

A. Cocktails have been optimized for the selection of human, mouse, rat and non-human primate samples.

CLUMPY SAMPLES

Q. ARE THERE ANY SUGGESTIONS FOR CLUMPY SAMPLES?

A. Yes. There are several reasons a sample might clump.

1) If the cells were frozen or poorly stored then DNA from dead cells can form clumps. If this is the case adding DNAse to the cell suspension may help.

2) The presence of Ca++/Mg++ in the medium may cause cells, such as monocytes, to clump. Ca++/Mg++ free PBS should be used. EDTA can also be used to decrease clumping.

3) Cells may clump if they become activated. Keeping the cells at 4°C or on ice can prevent this.

Q. MY CORD BLOOD SAMPLE IS CLUMPING. WHAT IS HAPPENING?

A. Cord blood can frequently be “clumpy”. Often, this is a platelet problem, so the slow spin procedure outlined in the StemSep™ Instruction Manual (pg. 33) should be followed.

Q. WHICH ANTICOAGULANT SHOULD BE USED?

A. ACD is better than heparin as it is less likely to give a clumpy or sticky cell suspension.