

## StemClear™\*

Density gradient system for purification of stem cells

*For clinical use only*

StemClear is sterilized by sterile filtration: **STERILE A**

Document reference: FP09 I44 R01 A.1, Update: 16/02/2006

### DESCRIPTION

StemClear is a density gradient system for the enrichment of hematopoietic stem cells out of bone marrow or cord blood for clinical use such as stem cell transplantation purposes.

StemClear gradient consist of silane-coated colloidal silica particles suspended in PBS buffer.

### MATERIAL INCLUDED WITH THE KIT

*Productcode: STC125*

1x 125mL StemClear solution

*Productcode: STC300*

1x 300mL StemClear solution

### PRODUCT SPECIFICATIONS

pH: 7.15 - 7.40

Osmolality: 295-310 mOsm/kg

Sterility: Sterile

Density: 1.080-1.082 g/mL

Endotoxin: < 0.25 EU/mL

Certificate of analysis available on request

### MATERIAL NOT INCLUDED WITH THE KIT

- Cell counter
- Weighing scale with a range up to 1000g
- Centrifuge able of centrifuging transfer bags of up to 150mL (cord blood) or 600mL (bone marrow)
- LAF bench
- Hand sealer
- Sterile docking device
- Refrigerator (2-8°C)
- Pipettes + tips
- Plasma extractor
- Adapters / Spikes for the bags
- Fenwall Plasma extractor
- Ampul 1.8mL
- Bactec plus + aerobic/F, Bactec plus + anaerobic/F
- Transfer bag of 150mL
- Tubes of 4.5mL and 15mL
- Kocher or clamp
- Needles + syringes (1, 2, 5, 10, 20 and 50mL)
- Rubber mats 9 by 11cm
- Sanadep swabs
- Tubing
- Dilution tube 400
- Tube connector (3-way)

### CALCULATION OF G-FORCES

The g-force of your centrifuge can be calculated using this formula:

$g = 1.118 \times r \times \text{rpm}^2$  or  $\text{rpm} = \text{Square root } \{g / (1.118 \times r)\}$

r = radius of centrifuge in mm

rpm = rotations per minute / 1000

e.g. 1

r = 100 mm                  rpm = 1800 rotations per minute

$g = 1.118 \times 100 \times 3.24 = 362g$

e.g. 2

r = 100 mm                  g = 350g

$\text{rpm} = \text{SQR } \{350 / (1.118 \times 100)\} = 1.77$

= 1770 rotations per minute

### INSTRUCTIONS FOR USE WITH CORD BLOOD

#### GENERAL

1. All actions have to be performed in a Class A biological LAF bench
2. Clean and disinfect the work bench prior to commencing the procedure
3. Cleaning and disinfecting should best be performed using a 70% alcohol solution and linen free cloth or any other type of disinfectant of the same potency

#### SAMPLE PREPARATION

1. Weigh the volume of the cord blood sample
2. Mix the cord blood in the sampling bag
3. Mix again with a 5mL syringe and needle via the extraction plug of the sampling bag by extracting and re-injecting the blood sample 5 times

4. Extract 2.5 mL from sampling bag:
  - a. 1 mL in a Bactec plus + aerobic/F bottle (label clearly as "before")
  - b. 1 mL in a Bactec plus + anaerobic/F bottle (label clearly as "before")
  - c. 0.5 mL in a 4.5mL tube. Pipette 0.4mL out of this tube into a dilution tube containing 0.4 mL Human Serum Albumin (20%) and determine the hematocrit

#### PREPARATION OF THE TRANSFERBAG WITH STEMCLEAR

1. Transfer 75mL of StemClear via a sterile tube out of the bottle into a transfer bag
2. Prepare as many bags as will be needed later based the following calculation:

$X = \text{volume} / 75$

Round of this number if the hematocrit is below 0.25/L, round up if the hematocrit is higher than 0.26/L

#### SUPPLEMENTATION OF THE CORD BLOOD

1. Weigh the collected amount of cord blood
2. Calculate the required amount of gradient bags based on the formula shown above
3. Supplement the cord blood with PBS/EDTA buffer to the desired volume

#### GRADIENT SEPARATION

1. Connect the transfer bag with the StemClear medium to an empty 150 mL transfer bag
2. Transfer 75mL of StemClear medium into a 150mL transfer bag. Repeat this for all the 150mL bags as calculated above
3. Place the transfer bag containing the StemClear medium into a centrifuge bucket
4. Slowly transfer 75mL of cord blood into the transfer bag without mixing the layers
5. Repeat this procedure for the total amount of cord blood (as many times as calculated above)
6. Centrifuge for 20 minutes at 700g
7. Transfer the centrifuged density gradient into a plasma extractor
8. Connect a 600 mL transfer bag to the gradient bag and place the empty transfer bag on the weighing scale
9. Transfer the layer with purified cells, plus an extra 10 grams, into the 600mL transfer bag
10. Repeat this procedure for each of the gradient bags thus collecting up to 2 centrifuged bags per 600mL transfer bag

#### WASHING

1. Supplement the transfer bag containing the purified cells up to 600mL with PBS/EDTA buffer
2. Centrifuge for 20 minutes at 500g
3. Connect an empty 600 mL bag to this bag and transfer the supernatant into a fresh 600mL transfer bag
4. Repeat this procedure for each of the centrifuged 600mL transfer bags

#### VOLUME REDUCTION

1. Connect an empty 150mL transfer bag to the 600mL transfer bag and transfer the purified cell pellet from the 600mL transfers bag to the empty 150mL bag
  2. Flush the 600mL bag with PBS/EDTA buffer and transfer this into the transfer bag
  3. Repeat the steps above if the total amount of bags of purified cells (as mentioned in step 10, gradient separation) is greater then 2
  4. Centrifuge for 10 minutes at 500g
  5. Place the centrifuged bag in the plasma extractor, connect an empty 600mL transfer bag and transfer the supernatant
  6. Supplement the sediment with PBS/EDTA buffer up to 22.1mL
- Note: If the volume of the pellet is greater than 22.1mL and smaller than 26.0 mL no further action is required. Repeat the volume reduction step if the pellet volume is lower than 26mL

7. Weigh the transfer bag containing the pellets and connect it to a 50mL cryopreservation bag
8. Transfer the cells into the cryopreservation bag and flush the 150mL transfer bag with PBS/EDTA buffer to a volume of 22.1mL (maximally 26mL)
9. Mix well
10. Sample 0.5mL out of the cryopreservation bag
  - a. Pipette 0.4mL in a dilution tube containing 0.4 mL Human Serum Albumin (20%)
  - b. Clearly identify the tube as "after"
  - c. Perform a nucleated cell count
11. Place the cryopreservation bag into the refrigerator (2-8°C) for at least 20 minutes

#### CRYOPRESERVATION

Perform the cryopreservation as described in your institutional standard operating procedures

## INSTRUCTIONS FOR USE WITH BONE MARROW

### GENERAL

1. All actions have to be performed in a Class A biological LAF bench
2. Clean and disinfect the work bench prior to commencing the procedure
3. Cleaning and disinfecting should best be performed using a 70% alcohol solution and linen free cloth or any other type of disinfectant of the same potency

### SAMPLE PREPARATION

1. Weigh and take note of the volume of the sample of bone marrow A
2. Take a 1mL sample and determine the amount of nucleated cells
3. Connect an empty 600mL transfer bag to the sample bag containing the bone marrow and transfer the bone marrow into this bag
4. Place the bag into the centrifuge and centrifuge for 20 minutes at 662g

### EXTRACTION

1. Remove the bucket with the bone marrow from the centrifuge
2. Connect an empty 600mL transfer bag to the centrifuged bag
3. Place the centrifuged bag into a plasma extractor
4. Transfer the plasma into the empty 600mL transfer bag down to approximately 1cm above the pellet
5. Repeat for bone marrow bag B

### PREPARATIONS FOR THE SEPARATION PROCEDURE

1. Connect a bag with PBS / EDTA / HSA buffer solution to the bag containing the pellet
2. Add buffer solution to a volume of 700mL
3. Mix the bag with the diluted bone marrow
4. Weigh again
5. Take a 0.5 mL sample from the bag using a needle and syringe and transfer into a counting tube
6. Measure the hematocrit
7. Write down to hematocrit and the weight of the bag and make the following calculation:

$$\text{Interphase} = \{ [(1 - \text{Ht}) \times \text{volume}] / 2 \} + 100$$

Ht: hematocrit in L/L (1 decimal) e.g. 0,18 L/L

Volume: Volume of the bag with bone marrow and buffer (approximately 700mL)

Interphase: the cell volume that needs to be collected

8. Place the 600mL transfer bag containing 300mL of StemClear medium into the centrifuge bucket and zero the weighing scale
  9. Using a 50mL syringe, slowly transfer 350mL diluted bone marrow on top of the StemClear layer and avoid mixing
- Note:** Mix the bag of diluted bone marrow before extracting content
10. Repeat this procedure with the other half of the diluted bone marrow using a second bag of StemClear

### CENTRIFUGATION

1. Centrifuge the transfer bag for 20 minutes at 700g

### STEM CELL COLLECTION

1. Gently remove the bag from the centrifuge and place it in the plasma extractor
2. Connect an empty 2 litre transfer bag to the centrifuged 600mL transfer bag containing the bone marrow / StemClear gradient and place the 2 litre bag on the weighing scale
3. Transfer the volume of purified cells as calculated in point 7 of the section "Preparations for the separation procedure" into the 2 litre transfer bag
4. Repeat, if necessary, the centrifugation and the subsequent steps for each bag containing bone marrow / StemClear

### WASHING PROCEDURE

1. Connect the 2 litre transfer bag to empty 600mL transfer bags and transfer the purified cells to the 600mL bags.
2. Rinse, if necessary, with PBS / EDTA / HSA buffer.
3. Centrifuge for 20 minutes at 500g
4. Place the centrifuged bags into the plasma extractor, connect an empty 600mL to the centrifuged bag and transfer the supernatant into the 600mL transfer bag
5. The cells from step 1 and 4 can now be used for further processing.

### STORAGE AND CONSERVATION

Store reagents at 2-8°C when not in use. Open and close bottles under aseptic conditions. The sterility of the contents of the bottles may be compromised once a bottle has been opened.

### WARNINGS AND PRECAUTIONS

All human, organic material should be considered potentially infectious.

Handle all specimens as if capable of transmitting HIV or hepatitis. Always wear protective clothing when handling specimens. StemClear does not contain antibiotics.

\*StemClear is a trademark of Clear Cell Technologies BVBA, Industriepark Noord 32, 8730 Beernem, Belgium

StemClear is produced by FertPro N.V., Industriepark Noord 32, 8730 Beernem, Belgium

