

StemSpan™ CC110

Serum-free culture supplement for expansion of human hematopoietic cells

Catalog #02697 1 mL



Scientists Helping Scientists™ | WWW.STEMCELL.COM

TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713

INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM

FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

StemSpan™ CC110 cytokine cocktail contains a combination of early-acting recombinant human (rh) cytokines formulated to support the proliferation of human hematopoietic cells. It is supplied as a 100X concentrate.

When added to serum-free medium, StemSpan™ CC110 promotes the expansion of CD34+ cells isolated from human cord blood (CB) and bone marrow (BM). StemSpan™ CC110 stimulates similar expansion of CD34+ cells compared to StemSpan™ CC100 (Catalog #02690), but with higher purity and is meant for use in short-term cultures to activate stem cell and immature progenitor cycling, without necessarily promoting the proliferation and differentiation of later progenitors.

We recommend using StemSpan™ CC110 in combination with any of the following StemSpan™ media:

- StemSpan™ SFEM (Catalog #09600)
- StemSpan™ SFEM II (Catalog #09605)
- StemSpan™-XF (Catalog #100-0073)
- StemSpan™-AOF (Catalog #100-0130)

Properties

Storage: Store at -20°C to -70°C.

Shelf Life: Stable until expiry date (EXP) on label.

Contains:

- Recombinant human fms-like tyrosine kinase 3 ligand (Flt3L)
- Recombinant human stem cell factor (SCF)
- Recombinant human thrombopoietin (TPO)

Directions for Use

PREPARATION OF COMPLETE MEDIUM

1. Thaw StemSpan™ CC110 at room temperature (15 - 25°C) or at 37°C until just thawed. Mix thoroughly.

NOTE: If necessary, centrifuge for 30 seconds to remove liquid from cap.

NOTE: If not used immediately, store at 2 - 8°C for up to 1 month. Alternatively, aliquot and store at -20°C. After thawing aliquots, do not re-freeze.

2. Add StemSpan™ CC110 to culture medium at a 1 in 100 dilution (e.g. add 1 mL of supplement to 99 mL of culture medium). Mix thoroughly.

RECOMMENDED PROTOCOL FOR CD34+ CELL EXPANSION WITH StemSpan™ MEDIA

For optimal performance, use one of the following StemSpan™ media in this protocol:

- StemSpan™ SFEM II (Catalog #09605)
- StemSpan™-AOF (Catalog #100-0130)

1. Thaw StemSpan™ medium at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.

NOTE: If not used immediately, aliquot into tubes and store at -20°C. Once aliquots are thawed, do not re-freeze.

2. Prepare complete medium as described in the Preparation section, using a StemSpan™ medium as the culture medium.

OPTIONAL: Add UM729 (Catalog #72332) to a final concentration of 1 µM; titration may be required to determine the optimal concentration for CD34+ cell expansion. UM729 enhances expansion of CD34+ cells, in addition to more primitive subsets such as CD34+CD90+CD45RA- cells.

3. Thaw cryopreserved CD34+ cells, or use an EasySep™ kit to isolate CD34+ cells from fresh whole CB or BM, or from cryopreserved CB or BM mononuclear cells (MNCs), as indicated below:
 - For fresh whole CB, use EasySep™ Human Cord Blood CD34 Positive Selection Kit II (Catalog #17896)
 - For fresh BM, or frozen BM or CB MNCs, use EasySep™ Human CD34 Positive Selection Kit II (Catalog #17856)
4. **Day 0:** Plate CD34+ cells in complete medium with or without UM729 (prepared in step 2). Refer to Table 1 for recommended plating concentrations; optimal cell concentrations and cultureware are dependent on experimental objectives and cell quality.

Table 1. Recommended CD34+ Cell Concentrations for Various Cultureware

CULTUREWARE*	VOLUME OF MEDIUM PER WELL	NUMBER OF CELLS PER WELL
6-well plate	2 mL	2×10^4
24-well plate	1 mL	1×10^4
96-well plate	100 μ L	1×10^3

*Both tissue culture-treated and non-tissue culture-treated are suitable.

5. Incubate at 37°C and 5% CO₂.
6. **Day 3 or 4:** Add an equal volume of fresh complete medium or perform a half-medium change.
7. **Day 7:** Harvest cells for evaluation or downstream applications. Count total viable cells using Trypan Blue (Catalog #07050) and a hemocytometer (or an automated cell counting method), and measure CD34+ expression by flow cytometry. Additional immunophenotyping may be performed to identify CD34+ cell subsets and/or differentiated CD34- cells.

NOTE: A 7-day culture period is optimal for cell yield, CD34 expression, and progenitor cell function. Shorter culture periods of 24 - 72 hours may be used if preserving CD34 expression and progenitor cell function is desired over high cell yield. Culturing beyond 7 days can be considered if high cell yields are desired. However, CD34 expression and progenitor cell function is reduced with longer culture times, due to cell differentiation.

If culturing for > 7 days: Cultures can be continued for an extended period of time with periodic dilution every 3 - 4 days to maintain a cell concentration < 1×10^5 cells/mL.

NOTE: Antigen expression on cultured cells may not be as predictive for determining non-differentiated status or lineage potential compared to antigen expression on CD34+ cells that have not been cultured. For example, primary CD34+ cells with low or undetectable CD38 expression (CD34+CD38- phenotype) are highly enriched for hematopoietic stem cells and primitive progenitor cells, but CD34+CD38- phenotype of cultured cells may not be as primitive.

ASSESSMENT OF HEMATOPOIETIC CELLS

Assessment of CD34+ cells before and after culture may be performed by flow cytometry using the following fluorochrome-conjugated antibody clones:

- Anti-Human CD34 Antibody, Clone 581 (Catalog #60013) or Clone 563 (Catalog #60119) or Clone 8G12 (Catalog #60121)
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018) or Clone 2D1 (Catalog #60123)
- Anti-Human CD38 Antibody, Clone AT-1 (Catalog #60131) or Clone HIT2 (Catalog #60014)
- Anti-Human CD90 Antibody, Clone 5E10 (Catalog #60045)

Notes and Tips

RELATED PRODUCTS

For related products, including specialized culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com/HSPCworkflow or contact us at techsupport@stemcell.com. For available fresh and cryopreserved peripheral blood, cord blood, and bone marrow products in your region, visit www.stemcell.com/primarycells.

PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED. FOR ADDITIONAL INFORMATION ON QUALITY AT STEMCELL, REFER TO WWW.STEMCELL.COM/COMPLIANCE.

Copyright © 2020 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, EasySep, and StemSpan are trademarks of STEMCELL Technologies Inc. All other trademarks are the property of their respective holders. While STEMCELL has made all reasonable efforts to ensure that the information provided by STEMCELL and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.