Product Manual

CytoSelect™ 96-Well Hematopoietic Colony Forming Cell Assay

Catalog Number

CBA-320	96 assays
CBA-320-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

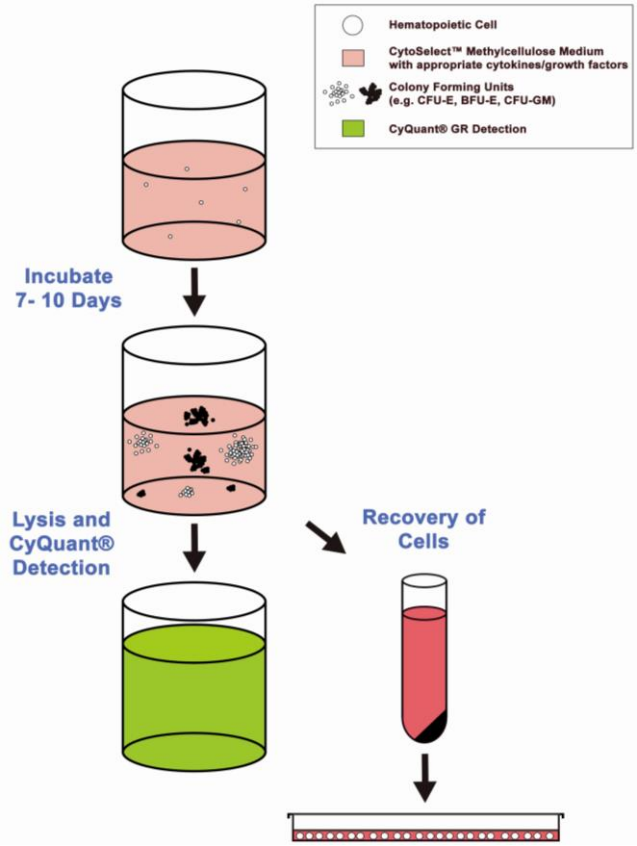
CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Hematopoietic stem cells (HSC) are well-characterized, tissue-specific stem cells that exhibit remarkable self-renewal capacity and are responsible for the life-long maintenance of the hematopoietic system. HSC are rare cells that reside in adult bone marrow where hematopoiesis is continuously taking place, but can also be found in cord blood, fetal liver, adult spleen and peripheral blood. Throughout the life span, hematopoiesis continuously replenishes the various lymphoid, myeloid and erythroid-megakaryocyte lineages, but also to maintain a small pool of HSC with the self-renewal capacity that is capable of carrying on hematopoiesis. From HSC to mature blood cells, extensive proliferation and expansion occurs that results in the production of millions of blood cells.

When cultured in a suitable semi-solid matrix, such as methylcellulose supplemented with nutrients and cytokines, HSC or hematopoietic progenitors called colony-forming cells (CFCs) proliferate to form discrete cell clusters or colonies. Colony types include colony-forming unit-erythroid (CFU-E), burst-forming unit-erythroid (BFU-E), CFU-granulocyte, macrophage (CFU-GM) and CFU-granulocyte, erythrocyte, macrophage, megakaryocyte (CFU-GEMM).

In classical CFC assays, culture usually takes place in a 35 mm dish for 14-21 days for the colonies to reach certain size (> 40 cells/colony) for manual counting. The CFCs are classified and manually enumerated based on the morphological recognition of one or more types of hematopoietic lineage cells within the colony.
Cell Biolabs' CytoSelect™ 96-well Hematopoietic Colony Forming Cell Assay does not involve subjective manual counting of colonies or require a 2–3-week incubation period. Instead, cells are incubated only 7-10 days in a semisolid methycellulose media before being solubilized, lysed and detected by the patented CyQuant® GR Dye in a fluorescence plate reader (see Assay Principle below). Alternatively, viable CFCs can be easily recovered for further culturing and testing. This format provides a quantitative, high-throughput method to accurately measure HSC or hematopoietic progenitor clonogenic capability.



Related Products

1. CBA-130: CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation)
2. CBA-140: CytoSelect™ 96-Well Cell Transformation Assay (Cell Recovery Compatible)
3. CBA-150: CytoSelect™ 96-Well In Vitro Tumor Sensitivity Assay
4. CBA-300: StemTAG™ Alkaline Phosphatase Staining Kit
5. CBA-301: StemTAG™ Alkaline Phosphatase Activity Assay Kit

Kit Components

1. 4X Lysis Buffer (Part No. 10404): One 10 mL bottle
2. CyQuant® GR Dye (Part No. 10105): One 75 µL tube
3. CytoSelect™ Methylcellulose Medium (Part No. 132001): One sterile 15 mL bottle of 1% Methyl-cellulose, 25% FBS, 2% BSA, 0.15% $NaHCO_3$, 50 µM 2-mercaptoethanol in Iscove's MDM

Materials Not Supplied

1. Cells and Culture Medium
2. Cell Resuspension Media (IMDM containing 25% FBS)
3. Cytokine/Growth Factor Supplements
4. 1X PBS
5. 96-well Tissue Culture Plate

Storage

Store the CytoSelect™ Methylcellulose Medium at -20ºC. If the methylcellulose medium will not be used all at once, it should be aliquoted to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- Thaw CytoSelect™ Methylcellulose Medium at 4ºC overnight. Mix the media well prior to aliquoting or use, ensuring a homogenous solution. Due to its viscosity, let the media to sit for 15 minutes to allow bubbles to rise to the top.

Assay Protocol

I. Hematopoietic Colony Forming Cell Assay (under sterile conditions)

1. Harvest cells in cell resuspension medium (see Materials Not Supplied) at 1 - 5 x 10^5 cells/mL. Desired cytokines and growth factors should be added directly to the cell suspension.

   Note: Cytokine/growth factors will be diluted 10-fold by addition of the CytoSelect™ Methylcellulose Medium.
2. Immediately dispense 15 µL of Cell Suspension containing growth factors into each well of a 96-well tissue culture plate.
3. Add 135 µL of CytoSelect™ Methylcellulose Medium (see Preparation of Reagents) to each well. Pipette each well 7-10 times to mix thoroughly.

Notes:

- *Try to avoid adding air bubbles to the well.*
- *To avoid fast and uneven evaporation that leads to aberrant results, we suggest not using the wells on the plate edge, or filling the edge wells with medium to reduce evaporation.*

4. Incubate the cells for 7-10 days at 37ºC and 5% CO_2. Examine the colony formation under a light microscope.

II. Quantitation of Colony Formation (skip to section III if cell recovery/re-plating is desired)

1. Prepare sufficient 4X Lysis Buffer/CyQuant® GR dye solution for all samples by diluting the dye 1:75 in 4X Lysis Buffer (for example, add 5 µL dye to 370 µL of 4X Lysis Buffer).
2. Add 50 µL of 4X Lysis Buffer/CyQuant® GR dye solution to each well (already containing 150 µL of solution). Pipette each well 7-10 times to ensure a homogeneous mixture. Incubate the plate at room temperature for 30 minutes.
3. Transfer 100 µL of the mixture to a 96-well plate suitable for fluorescence measurement.
4. Read the plate in a 96-well fluorometer using a 485/520 nm filter set.

III. Cell Recovery and Re-plating (under sterile conditions)

1. Add 150 µL of cold, sterile culture medium to each well.
2. Pipette each well 10-12 times to mix thoroughly.
3. Transfer the entire mixture to at least 20 volumes of standard culture medium (for example, 1 mL would be transferred to 20 mL media).

4. Centrifuge the cell pellet and aspirate the media supernatant.
5. Resuspend the pellet and transfer to a tissue culture flask or dish.
6. Transfer to a cell culture incubator.

Cell Dose Curve (Optional)

1. Harvest cells in cell resuspension medium at 1 - 5 x 10^6 cells/mL.
2. Prepare a serial 2-fold dilution in resuspension medium, including a blank without cells.
3. Transfer 150 µL of each dilution to a 96-well plate.
4. Add 50 µL of 4X Lysis Buffer/CyQuant® GR dye solution to each well (already containing 150 µL of solution). Pipette each well 7-10 times to ensure a homogeneous mixture. Incubate the plate at room temperature for 30 minutes.
5. Transfer 100 µL of the mixture to a 96-well plate suitable for fluorescence measurement.
6. Read the plate in a 96-well fluorometer using a 485/520 nm filter set.

Example of Results

The following figures demonstrate typical results with the CytoSelect™ 96-well Hematopoietic Colony Forming Cell Assay. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 485/538 nm filter set and 530 nm cutoff. One should use the data below for reference only. This data should not be used to interpret actual results.

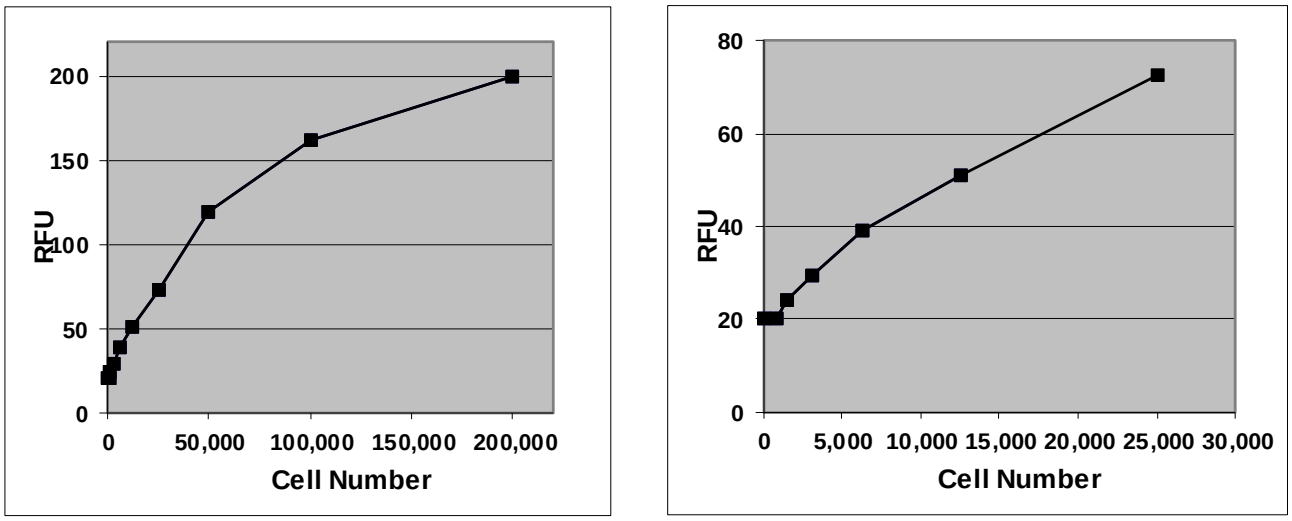


Figure 1: **CD34+ Cell Dose Curve**. Human bone marrow derived CD34+ Hematopoietic Progenitor Cells were resuspended at 2 x 10^6 cells/mL and titrated 1:2 in resuspension medium, followed by addition of Cell Lysis Buffer, and Cyquant® GR Dye detection (as described in the Cell Dose Section).

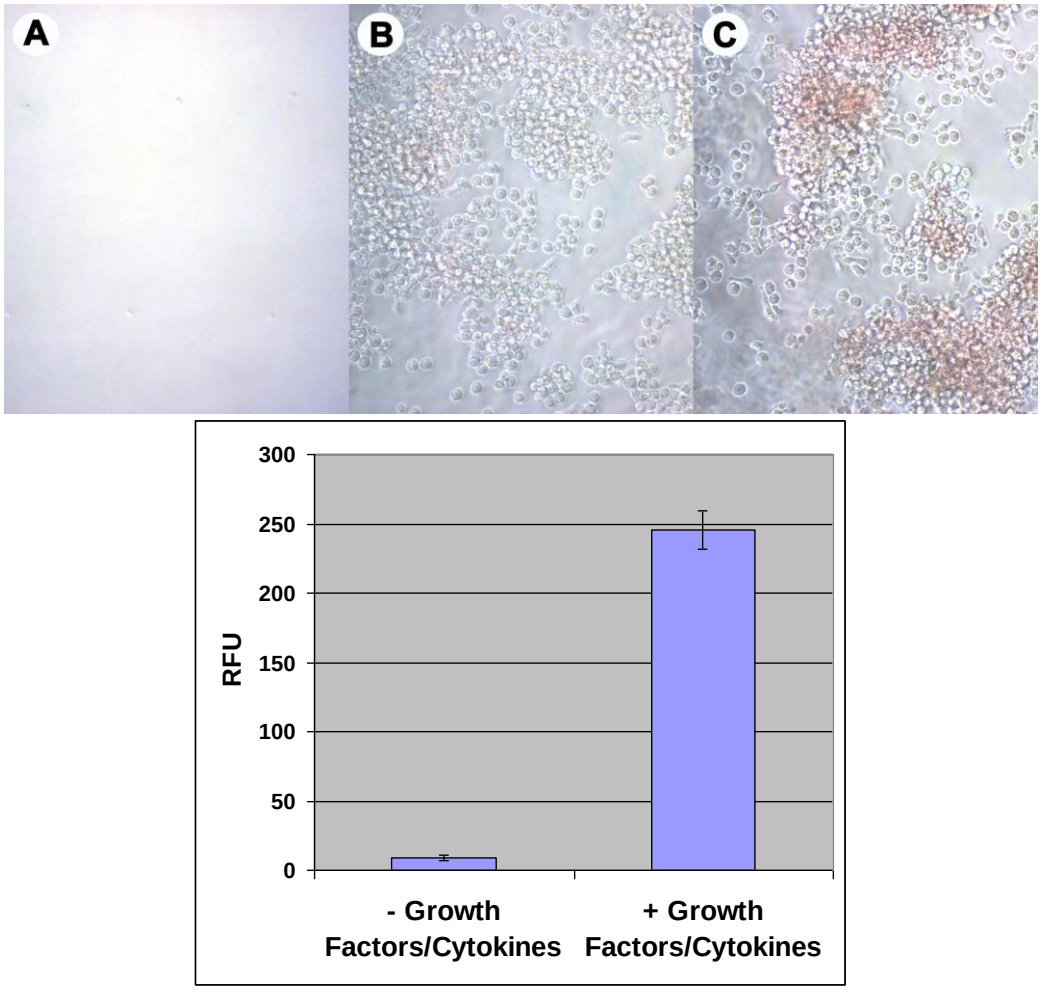


Figure 2: **Colony Formation**. Human bone marrow derived CD34+ Hematopoietic Progenitor Cells were seeded at 3000 cells/well and cultured for 7 days in the presence or absence of growth factors/cytokines (50 ng/mL SCF, 10 ng/mL hIL-3, 10 ng/mL hGM-CSF, 3 U/mL hEPO). Colony quantitation was determined according to the assay protocol. Photographs were taken after 7-day culture for Panel A (without growth factor/cytokine supplement) and Panel B (with growth factor/cytokine supplement). Panel (C) demonstrates growth after 10 days with growth factors/cytokines (hemoglobin clearly visible).

References

1. Ogawa, M. (1993) *Blood* **81**:2844.
2. Peters C, Steward CG (2003) *Bone Marrow Transplant* **31:**229-239.
3. Weissman, I.L. (2000) Science **287**:1442.
4. Ogawa, M. *et al*. (2002) Hematopoietic Stem Cell Protocol. Methods in Molecular Medicine. Humana Pres. p. 113.

Recent Product Citations

1. Nishi, Y. et al. (2019). Adipose tissue-derived mesenchymal stem cells ameliorate bone marrow aplasia related with graft-versus-host disease in experimental murine models. *Transpl Immunol*. pii: S0966-3274(18)30181-3. doi: 10.1016/j.trim.2019.03.004.

2. Singh, R. K. (2016). Alternative methods in haematopoietic stem cell toxicology. *Human Stem Cell Toxicology: Issues in Toxicology*: 9. doi:10.1039/9781782626787-00009.
3. Chiba, H. et al. (2013). Diabetes impairs the interactions between long-term hematopoietic stem cells and osteopontin-positive cells in the endosteal niche of mouse bone marrow. *Am J Phsiol Cell Physiol*. **305**:C693-C703.
4. Neri, P. et al. (2011). Bortezomib-induced "BRCAness" sensitizes multiple myeloma cells to PARP inhibitors. *Blood* **118**:6368-6379.

License Information

This product is provided under an intellectual property license from Life Technologies Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The sale of this product is expressly conditioned on the buyer not using the product or its components, or any materials made using the product or its components, in any activity to generate revenue, which may include, but is not limited to use of the product or its components: (i) in manufacturing; (ii) to provide a service, information, or data in return for payment; (iii) for therapeutic, diagnostic or prophylactic purposes; or (iv) for resale, regardless of whether they are resold for use in research. For information on purchasing a license to this product for purposes other than as described above, contact Life Technologies Corporation, 5791 Van Allen Way, Carlsbad CA 92008 USA or outlicensing@lifetech.com.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com