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ab242288 Hematopoietic Colony Forming Cell Assay Kit

View Hematopoietic Colony Forming Cell Assay Kit datasheet:
www.abcam.com/ab242288

For a quantitative, high-throughput method to accurately measure HSC or hematopoietic progenitor clonogenic capability.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

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.

The Hematopoietic Colony Forming Cell Assay Kit (ab242288) 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 GR Dye in a fluorescence plate reader (see Assay Procedure 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.

2. Protocol Summary

Prepare all reagents and samples as instructed.

⇩

Harvest cells in cell resuspension medium, add desired cytokines and growth factors. Immediately add 15 µL into each well of a 96-well plate.

⇩

Add 135 µL of Methylcellulose Medium to each well, mix well and incubate for 7-10 days at 37°C and 5% CO_2.

⇩

For quantification, prepare sufficient 4X Lysis Buffer/GR dye solution and add 50 µL to each well. Mix well and incubate for 30 minutes at RT.

⇩

Transfer 100 µL of the mixture over to a 96-well plate. Read the plate at 485/520nm.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at 4°C immediately upon receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage condition
4X Lysis Buffer	10 mL	4°C
GR Dye	75 µL	4°C
Methylcellulose Medium	15 mL	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Cells and Culture Medium
- Cell Resuspension Media (IMDM containing 25% FBS)
- Cytokine/Growth Factor Supplements
- 1X PBS
- 37°C Incubator, 5% CO2 Atmosphere
- Light Microscope
- 96-well Fluorometer
- 96-well Tissue Culture Plate

6. Reagent Preparation

- Thaw the Methylcellulose Medium at 4°C overnight. Mix the media well prior to aliquoting or use, ensuring a homogenous solution. Due to the viscosity, let the media sit for 15 minutes to allow bubbles to rise to the top.
- All other reagents are ready to use as supplied, warm to RT before use.

7. Assay Procedure

7.1 Hematopoietic Colony Forming Cell Assay (under sterile conditions):

7.1.1 Harvest cells in cell resuspension medium (see Materials Not Supplied) at 1 - 5 x 105 cells/mL. Desired cytokines and growth factors should be added directly to the cell suspension.

ΔNote: Cytokine/growth factors will be diluted 10-fold by addition of the Methylcellulose Medium.

7.1.2 Immediately dispense 15 µL of Cell Suspension containing growth factors into each well of a 96-well tissue culture plate.

7.1.3 Add 135 µL of Methylcellulose Medium (see Preparation of Reagents) to each well. Pipette each well 7-10 times to mix thoroughly.

ΔNote: Try to avoid adding air bubbles to the well.
ΔNote: 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.

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

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

7.2.1 Prepare sufficient 4X Lysis Buffer/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).

7.2.2 Add 50 µL of 4X Lysis Buffer/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.

7.2.3 Transfer 100 µL of the mixture to a 96-well plate suitable for fluorescence measurement.

7.2.4 Read the plate in a 96-well fluorometer using a 485/520 nm filter set.

7.3 Cell Recovery and Re-plating (under sterile conditions):

7.3.1 Add 150 µL of cold, sterile culture medium to each well.

7.3.2 Pipette each well 10-12 times to mix thoroughly.

7.3.3 Transfer the entire mixture to at least 20 volumes of standard culture medium (for example, 1 mL would be transferred to 20 mL media).

7.3.4 Centrifuge the cell pellet and aspirate the media supernatant.

7.3.5 Resuspend the pellet and transfer to a tissue culture flask or dish.

7.3.6 Transfer to a cell culture incubator.

7.4 Cell Dose Curve (Optional):

7.4.1 Harvest cells in cell resuspension medium at 1 - 5 x 106 cells/mL.

7.4.2 Prepare a serial 2-fold dilution in resuspension medium, including a blank without cells.

7.4.3 Transfer 150 µL of each dilution to a 96-well plate.

7.4.4 Add 50 µL of 4X Lysis Buffer/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.

7.4.5 Transfer 100 µL of the mixture to a 96-well plate suitable for fluorescence measurement.

7.4.6 Read the plate in a 96-well fluorometer using a 485/520 nm filter set.

8. Typical Data

The following figure demonstrates typical blot results for the CML-BSA Immunoblot Control. One should use the data below for reference only. This data should not be used to interpret actual results.

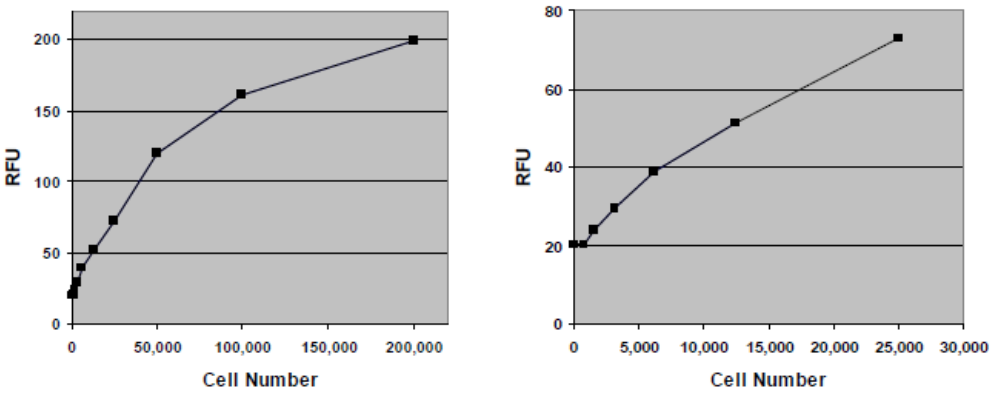

Figure 1. CD34+ Cell Dose Curve. Human bone marrow derived CD34+ Hematopoietic Progenitor Cells were resuspended at 2 x 10^6 cells/mL and titrated 1:2 in resuspension medium, followed by addition of Cell Lysis Buffer, and GR Dye detection (as described in the Cell Dose Section).

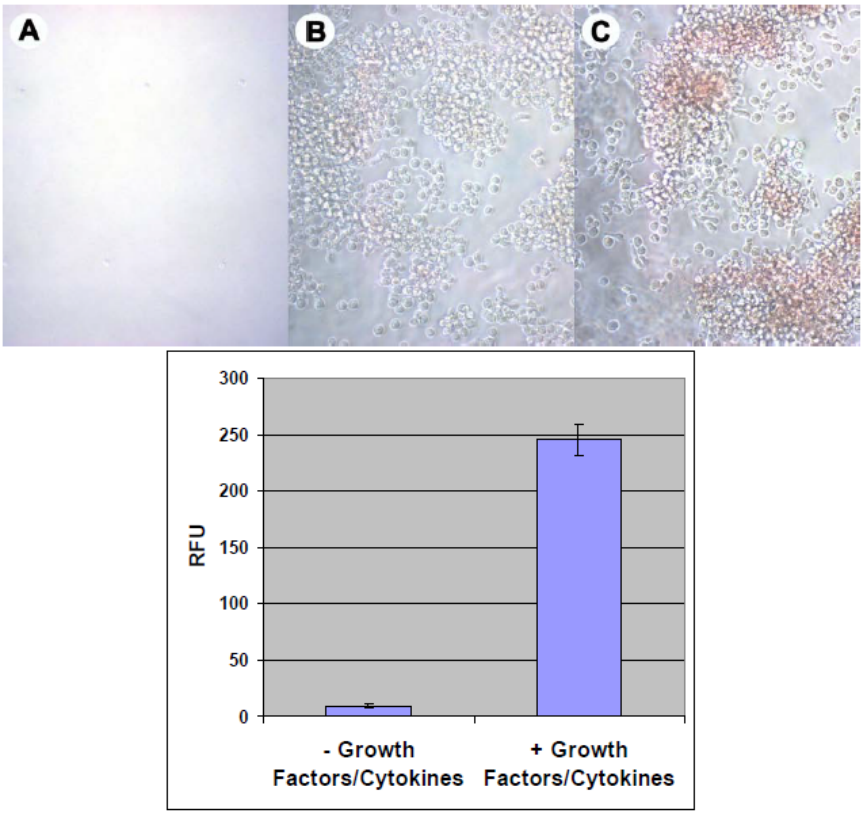

Figure 2. Colony Formation. Human bone marrow derived CD34+ Hematopoietic Progenitor Cells were seeded at 3000 cells/well and cultured for 7 days in the presence or absence of growth factors/cytokines (50 ng/mL SCF, 10 ng/mL hIL-3, 10 ng/mL hGM-CSF, 3 U/mL hEPO). Colony quantitation was determined according 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).

9. Notes

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