HemaTox™ Megakaryocyte Kit

Serum-free medium and supplement for the measurement of drug toxicity on megakaryocyte progenitor cells

Catalog #09707 1 Kit

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Product Description

HemaTox™ Megakaryocyte Kit is intended for the measurement of drug toxicity on the proliferation and megakaryocyte-specific differentiation of human hematopoietic stem and progenitor cells (HSPCs) in a liquid culture-based 96-well plate format.

HemaTox™ Megakaryocyte Kit includes a specialized serum-free culture medium and 100X supplement. Complete HemaTox™ Megakaryocyte Medium (HemaTox™ Megakaryocyte Medium + HemaTox™ Megakaryocyte 100X Supplement) promotes the proliferation of human CD34+ HSPCs and their differentiation into megakaryocytes during a 10-day culture period. After culture, the cells can be counted and assessed for expression of megakaryocyte marker CD41 using flow cytometry or other methods.

HemaTox™ Megakaryocyte Kit may be used on its own or in combination with HemaTox™ Myeloid Kit (Catalog #09704) or HemaTox™ Erythroid Kit (Catalog #09701) to assess lineage-specific drug toxicity in parallel.

Each kit contains sufficient medium and supplement for testing up to 160 different conditions (triplicate wells per condition, 200 µL per well) in 5 x 96-well plates.

Product Information

The following components are sold as a complete kit (Catalog #09707) and are not available for individual sale.

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE
HemaTox™ Megakaryocyte Medium*	09708	100 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
HemaTox™ Megakaryocyte 100X Supplement	09709	1 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

*This product contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
96-well flat-bottom plates	e.g. Corning 3596
245 mm x 245 mm Square Treated Tissue Culture Dishes	27140
35 mm Culture Dishes	27100
Solvent for test compound(s), e.g. dimethyl sulfoxide (DMSO)	--
10% Bovine Serum Albumin (BSA) in Iscove's MDM	09300
Iscove's Modified Dulbecco's Medium (IMDM)	36150
Trypan Blue	07050

Preparation of Reagents and Materials

Complete HemaTox™ Megakaryocyte Medium

Use sterile techniques to prepare **complete** HemaTox™ Megakaryocyte Medium (HemaTox™ Megakaryocyte Medium + HemaTox™ Megakaryocyte 100X Supplement). The following example is for preparing 20 mL of complete medium. If preparing other volumes, adjust accordingly.

NOTE: Prepare only the volume of **complete** HemaTox™ Megakaryocyte Medium required for a single experiment. A 20 mL volume is sufficient for one 96-well plate at 200 µL/well.

1. Thaw HemaTox™ Megakaryocyte Medium at 2 - 8°C overnight. Mix well.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C until the expiry date (EXP) as indicated on the label. After thawing the aliquots, use immediately. Do not re-freeze.
2. Thaw HemaTox™ Megakaryocyte 100X Supplement at room temperature (15 - 25°C) immediately prior to use. Mix well.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C until the expiry date (EXP) as indicated on the label. After thawing the aliquots, use immediately. Do not re-freeze.
3. Add 200 µL of HemaTox™ Megakaryocyte 100X Supplement to 19.8 mL of HemaTox™ Megakaryocyte Medium. Mix thoroughly.

Test Compound Solutions

1. Dissolve or dilute the test compound in an appropriate solvent, preferably to at least 1000X the concentration at which it will be tested in culture. This is the test compound stock solution. Different dilutions may need to be prepared depending on the solubility of the test compound and the required concentration range.

   NOTE: A stock solution concentration ≥ 1000X will ensure that the final solvent concentration in the culture will be ≤ 0.1%. If DMSO is the solvent, a DMSO concentration of ≤ 0.1% will not affect cell growth.
2. Prepare a 2X test compound solution by diluting the test compound stock solution (prepared in step 1) in **complete** HemaTox™ Megakaryocyte Medium.

   NOTE: Prepare sufficient volume of 2X test compound solution for replicate wells at 100 µL/well. Three replicate wells are recommended for each test compound.
3. Prepare a solvent control by diluting solvent in **complete** HemaTox™ Megakaryocyte Medium to the same concentration as the solvent in the 2X test compound solution.

Isolation of Fresh CD34+ Cells from Cord Blood or Bone Marrow

Drug toxicity assays should be performed using purified human CD34+ cells isolated from cord blood (CB) or bone marrow (BM). The use of unfractionated or minimally processed CB or BM is not recommended.

CD34+ cells can be isolated from whole CB using EasySep™ Human Cord Blood CD34 Positive Selection Kit II (Catalog #17896) or from BM mononuclear cells using EasySep™ Human CD34 Positive Selection Kit (Catalog #18056). Freshly isolated CD34+ cells can be used immediately or aliquots may be cryopreserved for later use.

Directions for Use

Please read the entire protocol before proceeding.

A. THAWING, WASHING, AND DILUTION OF CD34+ CELLS

1. Thaw the vial of cells quickly (within 2 minutes) in a 37°C water bath by swirling gently.
2. When the cells are almost completely thawed, wipe the outside of the vial with 70% ethanol or isopropanol.
3. Transfer cells to a 15 mL or 50 mL tube.
4. Slowly (dropwise) add wash medium (e.g. 1% BSA in Iscove's MDM) to the thawed cells to a final volume of 10 mL or 50 mL while gently swirling the tube (approximately 1 - 2 minutes). Invert the tube to mix.
5. Centrifuge the cell suspension at 300 x *g* for 10 minutes at room temperature (15 - 25°C).
6. Carefully remove the supernatant, taking care not to dislodge the cell pellet. Do not pour off.
7. Resuspend the cell pellet by gently flicking the tube. Add a known volume of either IMDM or **complete** HemaTox™ Megakaryocyte Medium.
8. Perform a viable cell count using Trypan Blue and a hemocytometer, or use an automated cell counter.

   NOTE: Methods to assay viable cells (e.g. dye exclusion) should be used for cell preparations where a decrease in cell viability may be expected (e.g. cryopreserved cells).

9. Dilute the cells in **complete** HemaTox™ Megakaryocyte Medium to a concentration of 2000 - 4000 viable CD34+ cells/100 µL (20,000 - 40,000 cells/mL).

   NOTE: Prepare at least 10 mL of cell suspension for each 96-well plate to ensure that there is a sufficient volume to seed the required number of wells for the experiment (100 µL/well).

B. PLATING

1. Mix the cell suspension (prepared in section A) immediately before use. Add 100 µL of the cell suspension to each well of the 96-well flat-bottom plate.
2. Add 100 µL of the appropriate 2X test compound solution or solvent control to each well.

   NOTE: Three replicate wells are recommended for each test compound.
3. Place each 96-well plate in a 245 mm x 245 mm Square Treated Tissue Culture Dish. Within this outer tissue culture dish, surround the 96-well plate with 4 x 35 mm Culture Dishes containing ~3 mL of sterile water.
4. Incubate at 37°C in 5% CO_2 and > 95% humidity for 10 days.

C. CULTURE ANALYSIS

Choose an appropriate analysis method for determining the effect of test compounds on HSPC proliferation and megakaryocyte differentiation.

We recommend labeling cells with antibodies for cell surface markers that characterize megakaryocytes, then counting cells that express the markers using a flow cytometer with absolute cell counting capability. Recommended antibodies are shown in Table 1.

Table 1. Recommended Antibodies for Labeling Megakaryocyte Cells

ANTIBODY*	CATALOG #	LABELED CELL POPULATIONS
Anti-Human CD45 Antibody, Clone HI30, PE	60018PE	Megakaryocytes (CD45+CD41+)
Anti-Human CD41 Antibody, Clone HIP8, FITC	60114FI	

*Recommended fluorochromes are shown; however, users should select the fluorochrome that is appropriate for their instrument and analysis.

Alternative analysis methods (e.g. automated cell counting, imaging cytometry, or plate reader-based methods) may also be used to quantify the response and obtain estimates for the 50% and 90% inhibitory concentrations (IC_{50} and IC_{90}) for each test compound in the assay. Optimization and validation may be required for each analysis method.

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, visit www.stemcell.com/primarycells.