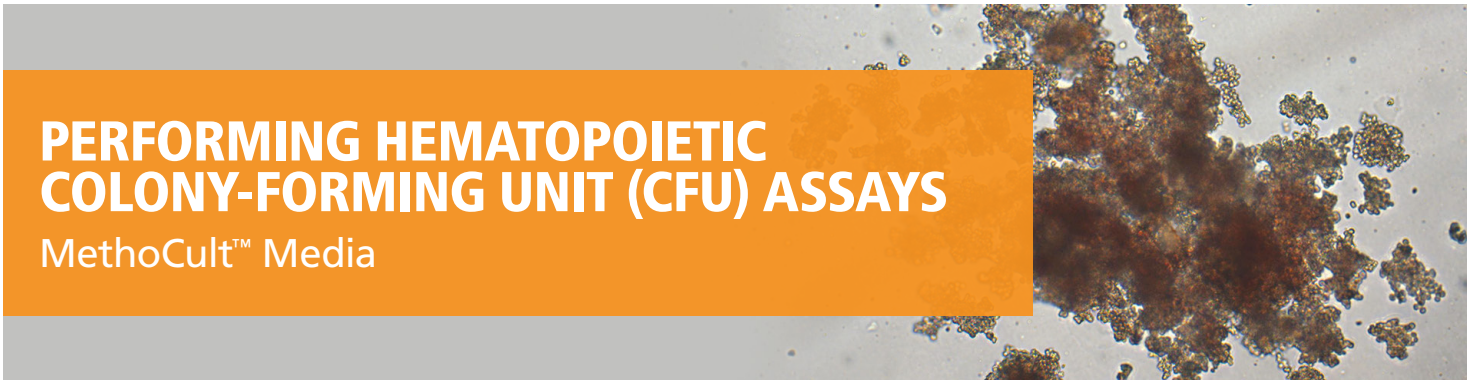
PERFORMING HEMATOPOIETIC COLONY-FORMING UNIT (CFU) ASSAYS

MethoCult™ Media



The CFU Assay

The colony-forming unit (CFU) assay is an in vitro functional assay for enumerating multipotential and lineage-committed hematopoietic progenitor cells (HPCs) in bone marrow, blood and other hematopoietic tissues. The CFU assay allows researchers to enumerate erythroid (CFU-E and BFU-E), granulocyte/macrophage (CFU-GM, CFU-G and CFU-M) and multilineage (CFU-GEMM) progenitor cells, as cells of different lineages and maturity produce colonies that differ in size, morphology and cellular composition. Each colony is derived from a single progenitor cell or CFU. MethoCult™ is a line of methylcellulose-based media formulated to promote the optimal growth and differentiation of HPCs from various species (humans, mice and rats). MethoCult™ media is a key component of standardized hematopoietic CFU assays. Pair MethoCult™ with SmartDish™ meniscus-free cultureware and STEMgrid™-6 for more accurate manual colony counting or perform automated counts of CFU assays in SmartDish™ cultureware with the STEMvision™ instrument. For those interested in improving their skills when performing the CFU assay, proficiency testing programs, quality control kits and training courses are available.

1 Step 1: Prepare Cells

- Red blood cells (RBCs) should be removed from samples prior to performing a CFU assay:
  - For mouse samples use ammonium chloride
  - For fresh human samples use HetaSep™
  - ErythroClear™ can be used to remove RBCs from multiple small samples of fresh or frozen cord blood
- Progenitors can be enriched with EasySep™, StemSep™, RosetteSep™ or FACS
- Prepare a cell suspension that is 10X the final plating concentration (e.g. 2 x 10^5 cells/mL for 2 x 10^4 cells/dish) by diluting with Iscove's MDM + 2% FBS
  Note: A 10X cell concentration should only be used if adding 300 µL of cells to 3.0 mL of MethoCult™ for duplicate dishes or 400 µL of cells to 4.0 mL of MethoCult™ for triplicate dishes

2 Step 2: Add Cells to MethoCult™ Medium

- Vortex tube to mix contents thoroughly
- Let stand for at least five minutes to allow bubbles to rise to the top

3 Step 3: Plate and Incubate

- Dispense MethoCult™ mixture into dishes for methylcellulose-based cultures using a blunt-end needle attached to a syringe
- Plating in meniscus-free SmartDish™ 6-well culture plates will improve the accuracy of colony counting
- Place the culture dishes in a large outer dish containing uncovered 35 mm dishes filled with sterile water to maintain proper humidity of MethoCult™ medium
- Incubate human cells for 7-14 days and mouse cells for 7-12 days in a humidified incubator at 37°C and 5% CO_2

4 Step 4: Count Colonies

- Manually, on an inverted microscope using SmartDish™ plates with STEMgrid™-6 attached
- Automatically, using a STEMvision™ instrument for imaging and counting CFU assays of human hematopoietic cells or mouse bone marrow

STEP 1 - Cell Sourcing & Isolation	
Primary Cells*	Fresh or cryopreserved human cord blood, peripheral blood and bone marrow
Ammonium Chloride Solution**	07800
Lymphoprep™**	07801
HetaSep™**	07806
ErythroClear™**	01739
EasySep™**	Immunomagnetic positive selection or enrichment
RosetteSep™**	Immunodensity enrichment
Iscove's MDM + 2% FBS	07700
MethoCult™ Cell Wash Medium***	87700
STEP 2 - Culture of HPCS	
MethoCult™ Express***	04437
MethoCult™ Optimum***	04034
MethoCult™ Optimum without EPO***	04035
MethoCult™ Enriched***	04435
MethoCult™ Enriched without EPO***	04535
MethoCult™ GF M3434	03434
MethoCult™ GF M3534	03534
MethoCult™ SF M3436	03436
Syringes	28230
Blunt-End Needles	28110
SmartDish™ 6-Well Plates	27370
35 mm Culture Dishes	27100
Large Outer Dishes	38039
STEP 3 - Analysis	
STEMvision™ Instrument	22000
STEMgrid™-6 Detachable Grid	27000
Gridded Scoring Dishes	100-0085

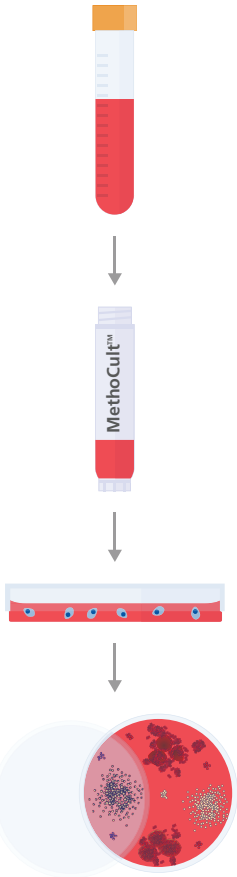
EPO: Erythropoietin

For detailed instructions, refer to our Technical Manuals for Human (Catalog #284040) and Mouse (Catalog #28405) Colony-Forming Unit (CFU) Assays Using MethoCult™.

Training Resources for the Hematopoietic CFU Assay*

Product	Catalog#
Human Bone Marrow Proficiency Testing Program	00602
Frozen Cord Blood Proficiency Testing Program	00608
Human Bone Marrow Quality Control Kit	00650
Human Cord Blood Quality Control Kit	00651
Hematopoietic Progenitor Assay Training Course	00215

*Certain products are only available in select territories. Please contact your local Sales representative or Product & Scientific Support at techsupport@stemcell.com for further information.
**Optional. Use of this reagent is dependent on institution's protocol for processing samples
***CE marked formulations also available for in vitro diagnostic use in the European Union (EU)



STEP 1
Prepare cells according to your institution's protocols.
Adjust cell concentration in Iscove's MDM + 2% FBS.

STEP 2
Add cells to MethoCult™.

STEP 3
Plate and incubate.

STEP 4
Count colonies.

EasySep™ Magnet


SmartDish™ 6-Well Plates


MethoCult™ Media in Tube or Bottle Formats


STEMvision™ Instrument

FREE WALLCHART

Human Hematopoietic Progenitor Wallchart
www.stemcell.com/humanCFUwallchart