

Serum-Free Medium Kit Without Growth Factors

Cat# NB-11-0065

Introduction

Kit consists of 500mL CSC Serum-Free Medium and 10mL Attachment Factor™. This is not a complete medium. For complete medium, add animal derived growth factor (CultureBoost) or human recombinant growth factor (RocketFuel). NB-11-0065 is Certified and intended for experimental application. CSC media and reagents are sterile, made with WFI and all components are cGMP and ISO Compliant.

Appropriate Use

Certified for use with more than 100 human and animal cell cultures and cell lines including these CSC and ACBRI Certified cells:

Human:

- Bone Marrow Cells (CFU-F and Erythroid Burst Cultures)
- Connective Tissue Cells
- Endothelial Cells (Aortic, Arterial, Coronary Artery, Vascular and Venous)
- Fetal Lung Cells
- Embryonic and Adult Stem Cells
- Fibroblast Cells
- Glomerular Mesangial Cells
- Immortalized / Tumor-derived Mesenchymal Cells
- Microvascular Cells (Cerebral, Coronary, Dermal, Glomerular, Liver, Lung and Retinal)
- Smooth Muscle Cells
- Tumor Neovascular Cells

Animal:

- Bovine and Porcine large vessel and microvessel Endothelial Cells

Additional Information

NB-11-0065 is also certified for ex vivo leukocyte expansion.

Characteristics of CSC Serum-Free Medium:

CSC Medium contains no added hormones

CSC Medium contains no antibiotics; however, Bac-Off® (Ciprofloxacin), Catalog #NB-11-0074, is the recommended antibiotic for all CSC Medium.

Use of Bovine Serum is no longer necessary. CSC Serum-Free Medium does not contain bovine-derived components. Human serum albumin and other components of human origin are specified by the formulation.

RocketFuel Supplement is supplied sealed in a non-oxidizing atmosphere. Plasma lipoprotein in RocketFuel is subject to oxidation upon contact with room air. The expiration date refers to unopened refrigerated storage of RocketFuel, the metric being inactivation of growth factor and oxidation of lipoprotein. Cellular binding of oxidized lipoproteins may be toxic to your cells. **Do not freeze** CSC Serum-Free Medium or RocketFuel supplements. The apoprotein is separated from lipoprotein by freezing, making binding to the receptor impossible, and may result in toxicity.

Special Requirements of the Serum-Free Environment: The *in vitro* requirements and culture procedures for cells employed in serum-free medium differ in several important ways from those appropriate for cells maintained in more traditional serum-containing cell culture. Use of Serum changes the phenotype of the cell. Information is transduced, processed, and physiologic response motifs are mediated by interaction with the paracellular microenvironment. Absent local or systemic injury, cells never “see” biologically active products of the clotting cascade and/or mediators of pathology produced by leukocyte degranulation. Among the significant benefits of Serum-Free cell culture is that, in principle, it is possible to eliminate artifacts resultant from indiscriminate cellular “response to injury”. CSC Serum-Free Medium was engineered from CSC Serum-Containing Medium, providing the components necessary for characterization, housekeeping metabolism, growth-factor dependent proliferation, and cell-specific experimental response.

Companion Products

Use of Attachment Factor is strongly recommended. Phenotypic and physiologic response motifs are mediated *in vivo* by cells from information transduced from the extracellular compartment. Culture of differentiated, polar, anchorage dependent cells in any medium requires attention to information processed by the cell from the extracellular matrix (ECM). Attachment Factor is an analog for the Natural ECM, provides information to the cell, and helps mediate these processes. **Phenol red is NOT included** in the formulation. Refer to the CSC Certificate for Attachment Factor.

Use of the Passage Reagent Group (PRG) is strongly recommended. Culture of fastidious cells in any medium requires careful formulation and calibration of reagents in order to detach cells for passage without excessive cytoskeletal and membrane damage. Trypsin is not “inactivated” by serum: the reduction of enzymatic activity on cell membranes is (at best) competitive and may be ineffective. Other enzymes (chymotrypsin, cathepsins, neutral proteases, etc.) not inactivated by serum in commercial (1:250) trypsin preparations make “inactivation” with serum during passage illogical. The PRG was engineered to meet the special challenges of modern cell culture, where thoughtful management of phenotype is important. Refer to the CSC Certificate for PRG.

Use of CSC Cell Freezing Medium is strongly recommended. Cell Freezing Medium is formulated using conditioned CSC Medium with DMSO as the principal cryoprotective agent. CSC Cell Freezing Medium is qualified by CSC and ACBRI for freezing cells whether cultured in serum-free or serum containing medium. Refer to the CSC Certificate for Cell Freezing Medium.

Handling and Storage

Medium Storage. Do not freeze Serum-Free Medium or RocketFuel Supplement.

Upon receipt, immediately refrigerate the medium and supplements. Once the unit is activated, or any component of the medium kit is opened, the shelf life is 30 days at refrigerated (4 - 8°C) temperatures.

Cell Storage

Remove the vial(s) from the dry ice shipping container and immediately transfer to liquid nitrogen. **ALWAYS store cells under liquid nitrogen.** Inapparent yet severe damage to membrane and cytoskeletal components results from chronic temperature fluctuations.

Additional Information

Thawing and Feeding Cells

- Activate CSC Serum-Free Medium with CSC RocketFuel™: each vial of RocketFuel activates 250mL Serum-Free Medium.
- Warm sufficient activated CSC Serum-Free Medium to 37°C in a water bath.
- Thaw the vial(s) of cells by immersing in a 37°C water bath. Observe carefully with gentle agitation and remove from the water bath just before the last of the ice disappears: this ensures that the cells are always kept close to the triple-point of water.
- Cleanse the vial(s) with 90% ethanol using a sterile 2X2.
- Immediately transfer the contents of the vial to at least 10 volumes of ice-cold Medium in a sterile centrifuge tube. Keep in an ice/water bath throughout to maintain triple-point temperature: cell viability is negatively affected by temperature excursions.
- Centrifuge 100-200 X g, 5-7 minutes refrigerated.
- Aspirate and discard supernatant. Leave 100-150 µl fluid to cover the pellet.
- Loosen the cell pellet by flicking with fingers.
- Count and adjust cell concentration at this time per your usual protocol.
- Prepare the new culture surface using Attachment Factor™.
 - Warm Attachment Factor™ to 37°C in the water bath.
 - Wet the culture surface to be inoculated with Attachment Factor™.
 - Aspirate and discard excess. Rinsing and/or drying are NOT necessary.

The culture may be inoculated at once.

- Re-suspend cells in CSC Complete Serum-Free Medium warmed to 37
- Seed on a tissue culture surface freshly coated with Attachment Factor.
- Incubate @ 37°C, 5% CO₂, 100% humidity.
- The vial of cells seeds a 75cm sq culture.

For Cell Passage

Refer to Passage Reagent Group™ instructions for more details.

- To feed, aspirate (and discard) spent medium. Add fresh CSC Serum-Free Medium.
- Feed at 12-24 hours and at least every 48 hours thereafter.
- As cultures approach confluence, daily feeding should be considered.