Human Normal and Asthmatic Airway Smooth Muscle Cell Lines

Product Document - 2014

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Human Normal and Asthmatic Airway Smooth Muscle (ASM) Cell Lines Panel

This overall panel has:

Asthma16 and Asthma26 (human ASM cells)

Normal10, Normal 11, Normal 12, and Normal27 (human ASM cells)

A subset of these cell lines are being offered for sale – individually or together in a bundled package.

The package can have:

Viable cells in T25 flasks have been sent to your laboratory – *especially useful in overseas shipments..*

Cryovials (one pair per cell line) have been sent to your laboratory – *use one to begin active culture and hold the other in cryopreservation for future use*

Prepared complete medium for both VascuLife SMC and Advanced MEM are provided – they are to be mixed 1:1 and filtered using the 1 Liter Filter Bottle System provided – DBM can continue to provide medium and medium additives to the purchaser if required – there is a surcharge for inclusion of this complete starter media.

Helpful Cell Culture Notes:

The cells are grown at 37 oC in a 5% CO2 gassed and humidified incubator.

The Asthma cell lines are hyperproliferative in DBM's hands versus the Normal cell lines.

The Asthma cell lines also have a stronger ASM cell morphology signature versus the Normal cell lines.

Within the Normal cell lines sub-panel, Normal10 and Normal11 proliferate more slowly than Normal12 and Normal27.

The cells were immortalized with a telomerase-based method involving introduction of hTert with a commercially available construct product.

The cells have been tested and are free of all pathogens.

DBM ASM Cell Medium Recipe

VascuLife medium (500 ml bottle) can be purchased from Lifeline Cell Technology – Cat# LM-0002.

The contents of a VascuLife SMC additive pack (Cat# LS-1040) are added to the VascuLife medium, along with 10 mls of standard Pen/Strep stock solution, 1 ml standard Fungizone (amphotericin B) stock solution and 150 ul standard Gentamicin stock solution.

Advanced MEM medium (500 ml bottle) can be purchased from ThermoFisher (Gibco-BRL, Life Technologies (Invitrogen) – Cat# 12492-013.

To the 500 ml bottle, add 25 mls of fetal bovine serum, 5 mls standard L-glutamine stock solution, 10 mls of standard Pen/Strep stock solution, 1 ml standard Fungizone (amphotericin B) stock solution and 150 ul standard Gentamicin stock solution.

Penicillin-Streptomycin (Pen-Strep) stock solution, Cellgro Mediatech, Cat#30-002-CI

L-Glutamine stock solution, Cellgro Mediatech, Cat#25-005-CI

Amphotericin B (Fungizone) stock solution, Cellgro Mediatech, Cat#30-003-CF

Gentamicin stock solution, Cellgro Mediatech, Cat#30-005-CR

Note: Cellgro Mediatech items can be purchased through ThermoFisher.

Again, mix the two 500 mls bottles and filter through the 1 Liter Filter Bottle Systems and use the DBM ASM Complete Cell Medium for culture and experiments.

DBM Freezing or Cryopreservation Medium

A freezing medium can be made with 500 mls Advanced MEM medium (from above and with additives) and by increasing FBS % to 15% (from 5%) and by adding 15% DMSO.

We also filter all of this medium with a 500 mls Filter Bottle System.

Thawing Cryovials for New Culture

A new practice is to simply thaw the cryovial in a 37 oC water bath and dilute it with standard culture medium (with added FBS to insure attachment) to seed flasks or microtiter plates directly – no centrifugation.

Replacement of the medium the next day or the day after that after inspection of cell attachment is recommended.

DBM recommends that the operator seed two T25 flasks initially – one can be used for further passage and experiments and one can be used to freeze down two cryovials for your laboratory at 500,000 cells each or more.

Passaging Cells

Our method is based on no or minimal trypsin-EDTA use.

(1) Remove medium by aspiration and wash 1X with CaMg-free PBS (Cellgro Mediatech, Cat# 21-031-CM).

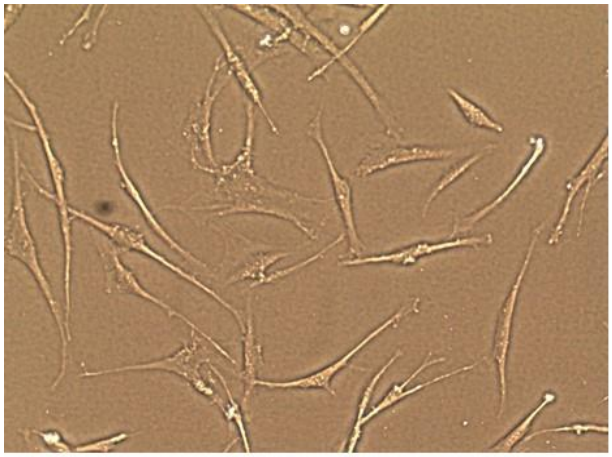
(2) Incubate for 30 minutes at 37 oC in a suitable volume of CaMg-free saline. Inspect the cells to see if they have loosened and agitate (spank) the flask (they may detach at this point). If yes, collect and add an equal volume of standard media and pellet by centrifugation.

DBM has observed that the human ASM cells will detach without trypsin-EDTA – it may require more than 30 minutes in CaMg-free PBS and frequent agitation (spanking) of the flask.

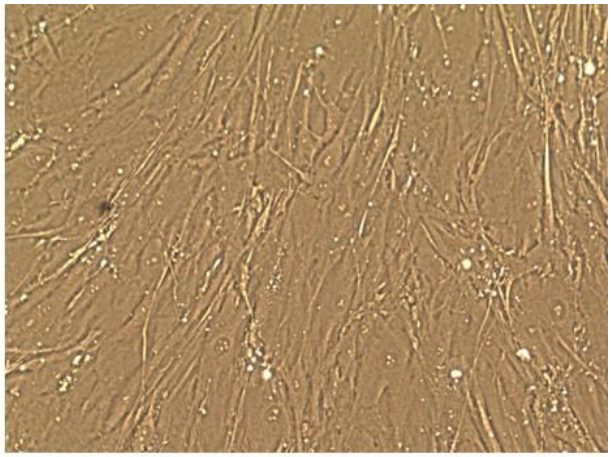
(3) Leave a bit of medium in the bottom of the tube, finger vortex to break up the pellet, resuspend in medium and seed the appropriate flasks or plates.

DBM recommends splitting with a 1:5 dilution for culture and bioassay/experimentation.

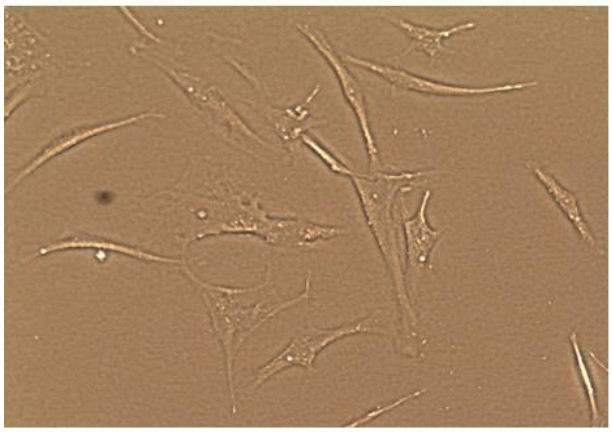
DBM recommends that the cells be studied in a passage window from passage 3/4 (number of passages after immortalization) through passage 10 to insure faithful results.



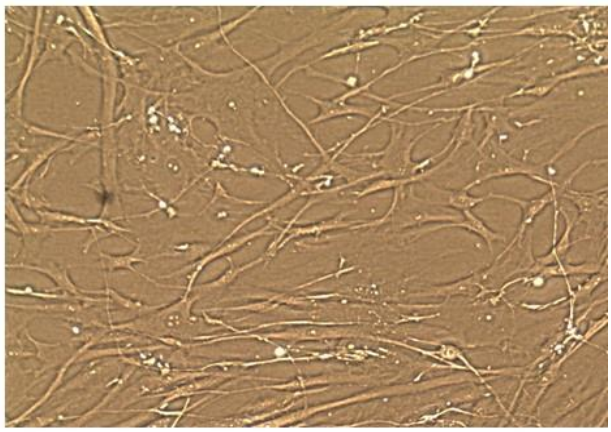
Asthma16 *Freshly Seeded*



Asthma16 *Confluence*



Normal27 *Freshly Seeded*



Normal27 *Confluence*

Please do contact Dr. Erik Schwiebert, Ph.D. at erik@discoverybiomed.com if you have further questions or if you wish to have a teleconference.

All the best of luck with our cells! EMS Dr. Erik M. Schwiebert, Ph.D. and the DBM Cell Culture Team