MRC-5 Conditioned Media for Primary Myoblast Cultures

Thaw a vial of MRC-5 cells and plate in a large (T75) flask in DMEM low glucose/10%FCS/1%pen/strep.
When semi confluent split 1:4 and grow until semi confluent.
Split again 1:4 for a total of 16 large flasks or 10cm plates and grow until 70% confluent.

Overlay cultures with F10/20% FBS/1% Pen/strep (10ml each plate).
Incubate cultures overnight, remove conditioned media, pool, pass through a 0.45um filter and aliquot into 5ml aliquots in 15ml tubes.
Store at –20C.
Replace media on MRC-5 cultures with fresh F10/20% FBS/1% Pen/strep and repeat once more.

To use conditioned media for myoblast propagation, thaw a tube just before use, add an equal volume of fresh F10/20% FBS/1% Pen/strep, and add 4ul bFGF and 10ul Dexamethazone/10 ml media.

Use 50% conditioned media to establish a new myoblast line until the first freeze down.

Note : Ensure that the cultures are not overgrown (70% is ideal) when beginning the overlay with F10/20% FBS/1% Pen/strep. A more confluent culture may release cell death proteins into the media by the second overlay.

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