Molecular Devices

Protocol

CloneMedia-CHOK1SV

Semi-solid media for the growth of CHOK1SV colonies

Control #: 03PCT1011.A0
Effective Date: 01-Jul-11
ECO #: 4002

Content

Storage

Store CloneMedia-CHOK1 SV at -20°C

Prior to use thaw CloneMedia-CHOK1 SV at 2°C – 8°C overnight. Do not shake contents until completely defrosted.

Allow to adjust to room temperature prior to using.

Once defrosted, CloneMedia-CHOK1 SV may be stored at 2°C – 8°C for up to one week.

For Research Use Only

Introduction

CloneMedia-CHOK1 SV is a complete animal-free semi-solid media for the growth of colonies of CHOK1SV cells.

Developed specifically for optimised use with Molecular Devices *ClonePix* mammalian colony picking systems, CloneMedia-CHOK1 SV supports colony formation as well as the fluorescent assay of secreted product using CloneDetect Agent.

Supplied as 90ml in 100ml bottles, supplements and selective agents can be added for optimal growth of your CHOK1 SV cell line without the need to aliquot.

Protocol

Please note:

The recommended plating procedures differ for transfected and stable cell lines.

Follow general instructions for **Media Preparation** (Part 1) first and then select and follow the appropriate instructions for **Cell Plating** (Part 2 for plating of stable CHOK1SV cell lines and Part 3 for the plating of recently transfected CHOK1SV cell populations).

1. Media Preparation

Semi-solid media prepared from 90ml of CloneMedia-CHOK1 SV is sufficient for the following;

Up to ten Molecular Devices PetriWell-1 Plates or 100mm tissue culture dishes (plate 9ml of media each)

Or

Up to eight PetriWell-6 Plates (plate 2ml of media per well).

Prior to use, thaw CloneMedia-CHOK1 SV at 2°C – 8°C overnight. Do not shake contents until completely defrosted. Allow to adjust to room temperature prior to use (do not pre-warm to 37°C). Prepare media from components adjusted to room temperature, only.

Defrosted CloneMedia-CHOK1 SV should be used as soon as possible, but can be stored at 2°C – 8°C for up to one week.

For 100ml of semi-solid media add any additional components necessary (e.g. selection agents, antibiotics) to the bottle containing 90ml of CloneMedia-CHOK1 SV. Bring up to a final volume of 100ml with sterile tissue culture water if necessary.

Should the volume of additional components exceed 10ml, ensure that the final volume of the media does not exceed 110ml.

Shake bottle vigorously for ca. 30 seconds to mix thoroughly. Allow time for bubbles to escape (ca. 10 minutes at room temperature). Any small bubbles remaining will disperse in the plate during the culture period.

Please select and follow **plating instructions applicable for your CHOK1 SV cell type** from the sections below.

2. Plating of stable transfected cell lines

It is recommended that conditions for successful plating are established with a stable transfected cell line before applying this to a recently transfected population where the window for selection of colonies is small.

Cell Lines can also be plated for analysis of the stability of the population or for re-cloning.

Methionine sulphozimine may be used at this stage if needed to maintain selection pressure. This is generally added at a concentration of 25µM to block the cells endogenous glutamine synthase activity.

For fluorescent detection assays, CloneDetect Agent may be added at this stage. Mix CloneDetect gently into the prepared media and protect bottle from direct light (Please refer to the CloneDetect protocol for detailed instructions).

Suspension-adapted CHOK1 SV cells growing in semi-solid conditions form discreet, spherical colonies suspended in the semi-solid media. (See Figure 1).

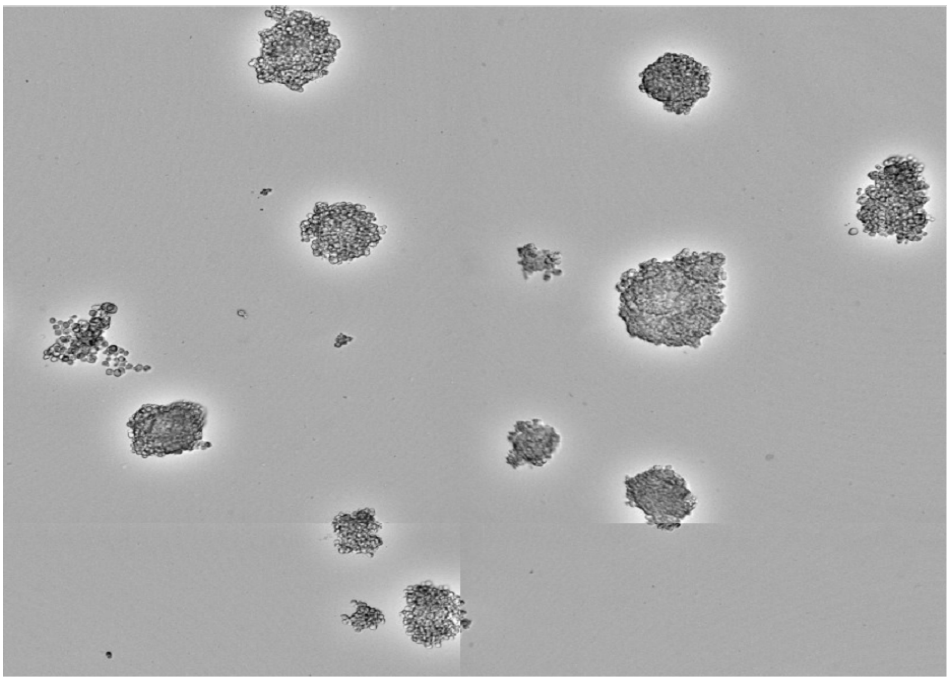

Figure 1: Images of colonies of CHOK1 SV cells grown in serum-free semi-solid media, taken on day 7 post-plating. Images were taken using Molecular Devices CloneSelect Imager.

The correct density of colonies in the culture dish is key to the success of colony selection and the automated picking process. It is therefore crucial to thoroughly **optimise the seeding densities.**

Seeding densities required to achieve an optimal density of colonies depend on the inherent plating efficiency of the cells used as well as the viability and growth phase of the cell suspension culture at the time of plating.

Cells should be plated 48-72 hours after their last passage and should have viability greater than 95%. Cells can be plated at a density of 300 - 750 viable cells/ml. Based on the density and viability of the cell suspension culture, as determined e.g. by haemocytometer count. Add the correct amount of cells to the semi-solid media and

gently invert several times to ensure even distribution.

To ensure clonality it may be necessary to dissociate cells by gently pipetting up and down the cell suspension but care must be taken not to damage the cells.

Allow the majority of bubbles to settle out by allowing the bottle to sit undisturbed for 2 minutes. Small bubbles will disperse in the plate during the culture period.

Dispense 2ml per well of a Molecular Devices PetriWell-6 Plate or 9ml of media per Molecular Devices PetriWell-1 Plate or 100mm tissue culture dish. This may be achieved using a 10ml pipette or by pouring.

Tilt the culture dish gently to ensure even distribution.

The incubation time required for CHOK1 SV in semi-solid media requires precautions to keep the semi-solid media well hydrated. To this end fill areas of the plate between wells with ca. 2-4ml of sterile water.

Place cultures in an incubator at 37°C, 5% CO2 for 7-14 days to allow colonies to grow.

Verify the presence of growing colonies at a suitable time point using a light microscope. Do not disturb plates from the incubator before day 4. At that time, small colonies of 25 to 100 cells should be visible. Avoid repeated checking of the plates. If necessary, have a specific imaging plate which will not form part of the main batch. This can be used to confirm the progress of colony growth and determine an appropriate window for picking.

3. Plating of transfected CHOK1SV (serum-free, suspension adapted CHO)

Prepare the media as described in Part 1. Up to 50µM methionine sulphoximine may be added to the media.

Do NOT add CloneDetect at this stage.

Due to the time required for selection using methionine sulphoximine, the seeding density varies according to the time the cells are in the selection media. Seeding cells into semi-solid media before seven days post-transfection is not recommended with cells in serum free or chemically defined conditions.

The optimum time for seeding is fourteen days post-transfection. Cells should be seeded at $2x10^3$ cells/ml to $5x10^3$ cells/ml. If plating before day 14, a higher seeding density will be required. Viability may still be low (ca. 50%) at this stage. This is not a concern.

Based on the density and viability of the cell suspension culture as determined e.g. by haemocytometer count, add the correct amount of cells to the semi-solid media and invert several times to gently to ensure even distribution.

Dispense 2ml per well of a Molecular Devices PetriWell-6 Plate or 9ml of media per Molecular Devices PetriWell-1 Plate or 100mm tissue culture dish. This may be achieved using a 10ml pipette or by pouring.

Tilt the culture dish gently to ensure even distribution.

The incubation time required for CHOK1SV in semi-solid media requires precautions to keep the semi-solid media well hydrated. To this end fill areas of the plate between wells with ca. 2-4ml of sterile water.

Place cultures in an incubator at 37˚C, 5% CO_2 for 10-14 days to allow colonies to grow.

Verify the presence of growing colonies at a suitable time point using a light microscope. Do not disturb plates from the incubator before day 4. At that time, small colonies of 2 to 16 cells should be visible. Avoid repeated checking of the plates. If necessary, have a specific imaging plate which will not form part of the main batch. This can be used to confirm the progress of colony growth and determine an appropriate window for picking.

If using CloneDetect to identify high antibody producing cells, this should be added by atomizer (K8201) at day 10. Addition at this stage prevents an accumulation of fluorescence around transiently expressing cells.

If by day 14, the colonies are still too small for picking (less than $0.5mm^2$ in area as judged by *ClonePix* software), the cells may continue to be incubated for up to an additional four days with no adverse affects on cell viability.

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Revised July 2011