MOLECULAR DEVICES

PROTOCOL

# CloneMedia-HEK

## Semi-solid media for the growth of HEK 293 colonies

Control #: 03PCT1012.A0
Effective Date: 01-Jul-11
ECO #: 4002

# Content



# Storage

Store CloneMedia-HEK at -20°C

Prior to use thaw CloneMedia-HEK 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-HEK may be stored at 2°C – 8°C for up to one week.

**For Research Use Only**

# Introduction

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

Developed specifically for optimised use with Molecular Devices ClonePix mammalian colony picking systems, CloneMedia-HEK 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 HEK cell line without the need to aliquot.

# Protocol

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

## 1. Media Preparation

Semi-solid media prepared from 90ml of CloneMedia-HEK 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-HEK 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-HEK 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-HEK. 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.

## 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 recloning.

**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).**

HEK 293 cells growing in semi-solid conditions form discreet, spherical colonies suspended in the semi-solid media. (See Figure 1).

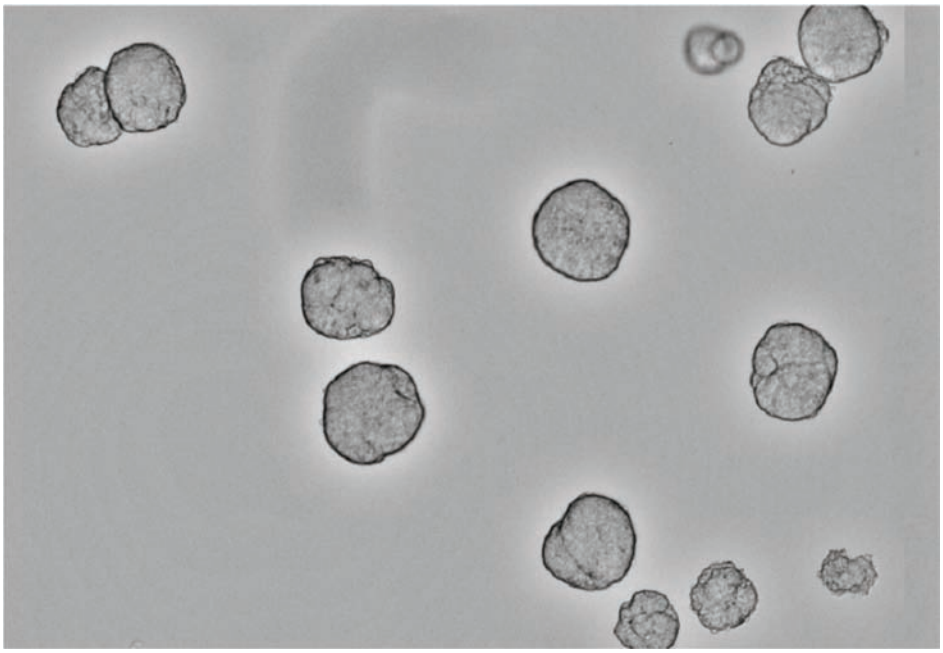

***Figure 1.*** *Images of colonies of HEK cells grown in animal-free semi-solid media, taken on day 11 post-plating. Images were taken using Molecular Devices CloneSelect Imager.*

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 1000 – 1500 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 HEK 293 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 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.

For all technical queries please contact your nearest Customer Support group.
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