CloneMedia™ Reagent

Table 1-1: Available CloneMedia Reagent

Item	Quantity	Part Number
CloneMedia Semi-Solid Media for Hybridoma and Myeloma cells, 90 ml	1 bottle	K8610
CloneMedia Semi-Solid Media for Hybridoma and Myeloma cells, 90 ml	6 bottles	K8600

The CloneMedia™ reagent provides semi-solid media for the growth of hybridoma and myeloma cells.

Contents

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Product manufactured by Molecular Devices, LLC.
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Molecular Devices, LLC is ISO 9001 registered.

Chapter 1: About the CloneMedia Reagent

CloneMedia reagent is a complete semi-solid media designed for the growth of colonies of mouse hybridoma and myeloma cells.

CloneMedia reagent is optimized for use with a ClonePix system and CloneSelect Imager label-free white light imaging system. The key advantage of using a ClonePix system and semi-solid culture is that the hybridoma and myeloma colonies in a population can be screened for productivity before clone isolation. CloneMedia reagent supports the growth of single cells into discrete, clonal colonies that are subsequently followed by automated isolation on a ClonePix system. The media is free of extraneous particulates providing exceptional optical quality of discrete colonies (Figure 3-1 A).

Since fluorescent screening of antibody-secreting cell lines requires colonies to be raised in semi-solid media, CloneMedia reagent provides suitable conditions for colony formation and facilitates fluorescent assays (for example, using CloneDetect agent) as the secreted product is immobilized in semi-solid media and visualized in the vicinity of the producing colony. Screening and isolation of high-secreting colonies is enabled by a ClonePix system, while the CloneSelect Imager conveniently provides high-speed label-free imaging to monitor cell growth and verify monoclonality. The ability of the ClonePix system to screen and rank secreting colonies before isolation significantly reduces the cell-line development timeline and maximizes monoclonality verification from the beginning of the process.

CloneMedia reagent contains colony promoting growth supplements and it is supplied without selective agents such as HAT. CloneMedia reagent is equally suitable for fresh fusion/transfections as well as for stable cell lines.

The product is conveniently supplied as 90 ml in 100 ml bottles eliminating the need to aliquot upon addition of selective agents and supplements. This prevents the risk of contamination allowing users to focus on the results steadily maintaining experimental workflow.

Chapter 2: Materials and Equipment

Package Components

Table 2-1: Available CloneMedia Reagent

Item	Quantity	Part Number
CloneMedia Semi-Solid Media for Hybridoma and Myeloma cells, 90 ml	1 bottle	K8610
CloneMedia Semi-Solid Media for Hybridoma and Myeloma cells, 90 ml	6 bottles	K8600

The entire 90 ml bottle of CloneMedia semi-solid media for hybridoma and myeloma cells (K8610) is sufficient to for eight (8) PetriWell-6 Plates (2 ml of media per well) or five (5) NUNC Omnitrays (18 ml of media per tray).

Storage and Handling

Store CloneMedia at –20°C. The product is stable for 1 year from date of shipment when stored under recommended conditions.

Before use, thaw CloneMedia at 2°C to 8°C overnight. Do not shake contents until completely defrosted. Allow to adjust to room temperature before using. When defrosted, CloneMedia can be stored at 2°C to 8°C for up to one week.

WARNING! Reagents can contain chemicals that are harmful. Exercise care when handling reagents as described in the related safety data sheet (SDS or MSDS). The safety data sheet is available in the Knowledge Base on the Molecular Devices support web site: www.moleculardevices.com/support.html

Chapter 3: Assay Protocol

Media Preparation

Before use:

- Thaw the CloneMedia reagent at 2°C to 8°C overnight.
- Do not shake contents until completely defrosted.
- Do not pre-warm to 37°C. Allow contents to adjust to room temperature before use.
- Prepare media from components adjusted to room temperature only.

Note: CloneMedia reagent can appear slightly opaque due to the high percentage of FBS. On rare occasions, slight variations in color can also occur. These factors do not interfere with either cell health/proliferation or image quality and analysis.

For 100 ml of semi-solid media:

1. Add any additional components necessary, such as selection agents or antibiotics, to the bottle containing 90 ml of CloneMedia reagent.
2. If necessary, add sterile tissue culture water to raise to a final volume of 100 ml.
   Allow space for the CloneDetect agent and a cell volume that will be added in subsequent steps.

To mix thoroughly:

1. Shake the bottle vigorously for approximately 30 seconds.
2. Allow time for the bubbles to escape, approximately 10 minutes at room temperature.
   Any remaining small bubbles will disperse in the plate during the culture period.

For fluorescent detection assays:

1. CloneDetect agent can be added at this stage.
2. Mix CloneDetect agent gently into the prepared media and protect the bottle from direct light. For detailed instructions, see the protocol for the CloneDetect agent.

Instructions for Cell Plating

Hybridoma cells growing in semi-solid conditions form discrete, spherical colonies suspended in the semi-solid media. (Figure 3-1).

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 optimize 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.

The following ranges of seeding densities can serve as a guideline for your optimization:

- Stable and robust hybridoma cell lines (100 to 400 cells/ml)
- Fresh fusions ($1x10^4$ to $1x10^6$ cells/ml)

Note: When plating fresh hybridoma fusions, the optimal seeding density is highly dependent on the fusion efficiency as well as the kinetics and efficiency of selection. For the successful selection of hybrids, the selection pressure applied, for example, concentration of HAT, can also require optimization. It is also highly recommended to allow the cells a period of 48 to 72 hours recovery in selection medium post-fusion, to allow unfused cells to die. The cells can be centrifuged gently and resuspended in an appropriate volume of medium before addition to the CloneMedia reagent.

1. Add the correct amount of cells to the semi-solid media. Invert the tube several times to gently distribute the cells evenly throughout the semi-solid media.
2. Dispense approximately 2 ml per well into a PetriWell-6 Plate or 18 ml per tray into an Omnitray using a pipette (for example, 10 ml) or by pouring.

   CAUTION! Only plate into non TC-treated culture dishes.
3. Tilt the culture dish gently to ensure even distribution.
4. Dispense 4 ml of sterile water into the area between the wells to ensure that the semi-solid media remains well hydrated.
5. Place the cultures in an incubator at 37°C, 5% CO2 for 5 to 10 days to allow the colonies to grow.

   CAUTION! Do not disturb the plates in the incubator before day 4 to allow small colonies to be visible.

6. Verify the presence of growing colonies at a suitable time point using a light microscope. For freshly-fused hybridoma plates, a large number of single cells might be visible (Figure 3-1); these are unfused cells that are non-viable and cannot divide.

CAUTION! Avoid repeated plate checking. If necessary, have a specific imaging plate that 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.

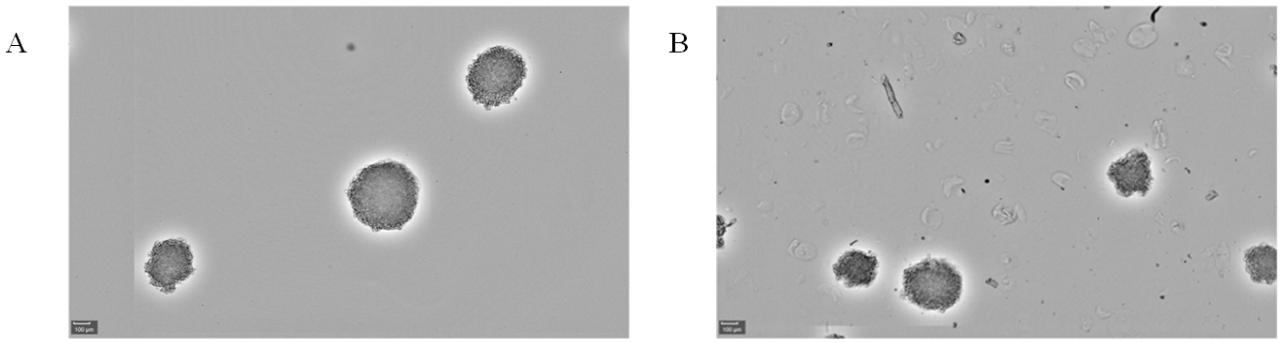

Figure 3-1: Images captured with Molecular Devices CloneSelect Imager of 123-10 colonies (stable cell line) at day 7 grown in A) Molecular Devices CloneMedia Hybridoma/Myeloma semi-solid media as compared to Product X from a different supplier (B). Colonies grown in Molecular Devices CloneMedia reagent exhibit exceptional optical clarity of distinct colonies.

Chapter 4: Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest possible level of technical service.

Our support web site, www.moleculardevices.com/support.html , has a link to the Knowledge Base with technical notes, software upgrades, safety data sheets, and other resources. If you do not find the answers you are seeking, follow the links to the Technical Support Service Request Form to send an email message to a pool of technical support representatives.

You can contact your local representative or contact Molecular Devices Technical Support by telephone at 800-635-5577 (U.S. only) or +1 408-747-1700. In Europe call +44 (0) 118 944 8000.

Please have the product name, part number, and lot number available when you call.

Contact Us

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Check our website for a current listing of worldwide distributors.

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