CloneMedia™ and XP Media™ CHO Growth A Reagents

Table 0-1: Available Reagents

Item	Quantity	Part Number
CloneMedia CHO Growth A with L-Gln, 90 ml	1 bottle	K8810
CloneMedia CHO Growth A with L-Gln, 90 ml	6 bottles	K8800
CloneMedia CHO Growth A without L-Gln, 90 ml	1 bottle	K8840
CloneMedia CHO Growth A without L-Gln, 90 ml	6 bottles	K8830
XP Media CHO Growth A, 1000 ml	1 bottle	K8860

Molecular Devices has partnered with Irvine Scientific to develop a complete CHO media portfolio comprising liquid and semi-solid media to ensure consistent cell-maintenance conditions throughout all stages of cell line development and antibody discovery workflows.

Contents

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Chapter 1: About the CloneMedia and XP Media CHO Growth A Reagents

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The CloneMedia™ and XP Media™ CHO Growth A reagents provide consistent media formulations throughout all stages in cell line development and antibody discovery workflows.

Molecular Devices has partnered with Irvine Scientific to develop a complete CHO media portfolio comprising liquid and semi-solid media to ensure consistent cell-maintenance conditions throughout all stages of cell-line development and antibody-discovery workflows. The media are chemically defined and animal component-free, have superior optical qualities, and are optimized for high-protein production allowing isolation of high producers early in the cloning process.

Recommended CHO Media for Cell Line Development and Antibody Discovery Steps

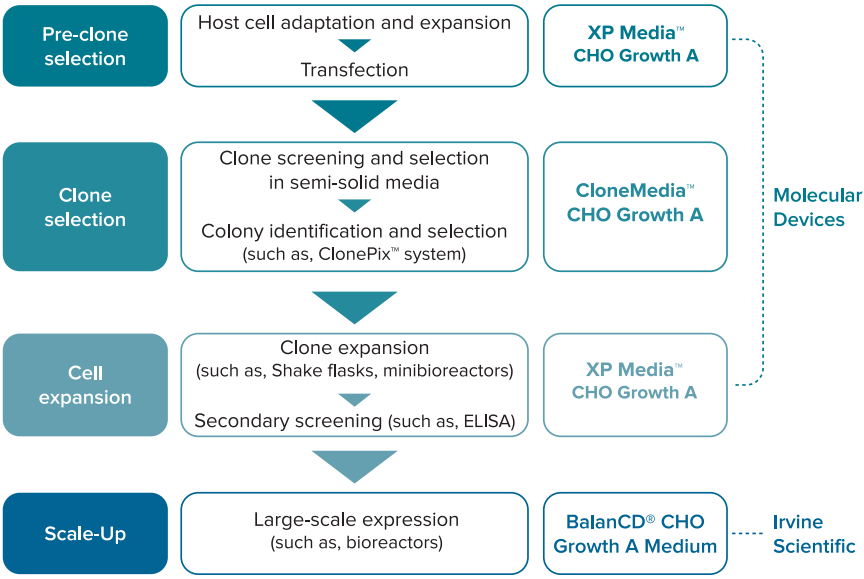

Figure 1-1: Recommended media types to support all stages of cell line development and antibody discovery workflows.

The complete CHO Media portfolio together with industry-proven ClonePix and CloneSelect Imager instruments improve the efficiency of isolating high-value clones.

BalanCD is a registered trademark of Irvine Scientific.

CloneMedia and XP Media CHO Growth A reagents are optimized for use with the ClonePix™ system and the CloneSelect™ Imager from Molecular Devices. Screening and isolation of high-secreting colonies is enabled by the ClonePix automated cell line development platform, while the CloneSelect Imager conveniently provides high-speed and label-free imaging tailored to monitor cell growth and verify monoclonality.

The key advantage of combining the ClonePix system and a three-dimensional, semi-solid cell culture environment is that a large and diverse population of CHO clones can be rapidly screened for protein production, picked, and directly deposited into microplates containing liquid media to facilitate cell expansion and further characterization by downstream assays. This is all accomplished with an automated work-cell approach.

CloneMedia CHO Growth A and XP Media CHO Growth A are chemically defined (CD), animal component-free (ACF) reagents, ensuring their compatibility for use in cell line development and bioprocessing applications. These media are HT (hypoxanthine, thymidine) deficient, so they are suitable for use with dihydrofolate reductase (DHFR) selection systems. Media are also available without L-Glutamine, allowing compatibility with the glutamine synthase (GS) selection system. See Table 1-1.

Table 1-1: Media Selection Guide

Item	Part Numbers	CD	ACF	HT	L-Glutamine
CloneMedia CHO Growth A with L-Gln	K8810 and K8800	✓	✓	–	✓
CloneMedia CHO Growth A without L-Gln	K8840 and K8830	✓	✓	–	–
XP Media CHO Growth A	K8860	✓	✓	–	–

CloneMedia CHO Growth A

Since fluorescent screening of antibody-secreting cell lines requires colonies to be grown in semi-solid media, CloneMedia CHO Growth A 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 immediate vicinity of the producing colony. The media is free of extraneous particulates, providing exceptional optical quality of discrete, rounded colonies. See Figure 1-2.

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 CHO Growth A reagent is superior to competitive products for media quality, colony growth, and antibody productivity.

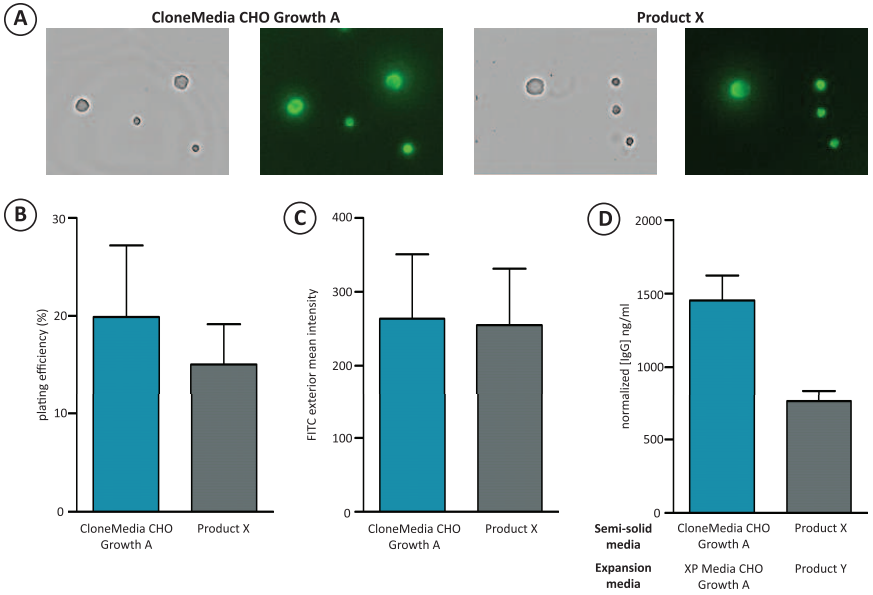

Figure 1-2: Suspension Adapted B13-24 Cells

Item	Description
A	Images captured with the Molecular Devices CloneSelect Imager (bright field) and ClonePix2 (FITC) instruments after 12 days growth in CloneMedia CHO Growth A reagent with L-Gln (K8810) or Product X from a different supplier. IgG secretion was detected using CloneDetect Human IgG, fluorescein (K8200).
B	Plating efficiency calculated using the CloneSelect Imager system Loci Count feature.
C	Mean fluorescence intensity of colonies following imaging on a ClonePix 2 Clone Picking System
D	Antibody productivity determined by ELISA of clone supernatants 10 days after pick, comparing media products from Molecular Devices with those of an alternative supplier. Values are normalized to 100% confluence.

CloneMedia CHO Growth A reagent contains colony-promoting growth supplements and is supplied either with or without L-Glutamine, and without selective agents. These options provide flexibility for use with multiple cell types and selection systems. CloneMedia CHO Growth A reagent is equally suitable for fresh transfections and for stable cell lines.

The products are conveniently supplied as 90 ml in 100 ml bottles, eliminating the need to aliquot upon addition of selective agents and supplements. This approach prevents the risk of contamination, letting you focus on the results and maintaining the experimental workflow.

XP Media CHO Growth A

XP Media CHO Growth A reagent supports the recovery and expansion of mammalian cells following colony picking from ClonePix systems. See Figure 1-3.

XP Media CHO Growth A is supplied without L-Glutamine or selective agents. XP Media CHO Growth A reagent is designed to maximize antibody productivity. Compared to competitive products, XP Media CHO Growth A reagent supports better post-pick growth, while maintaining high-antibody productivity.

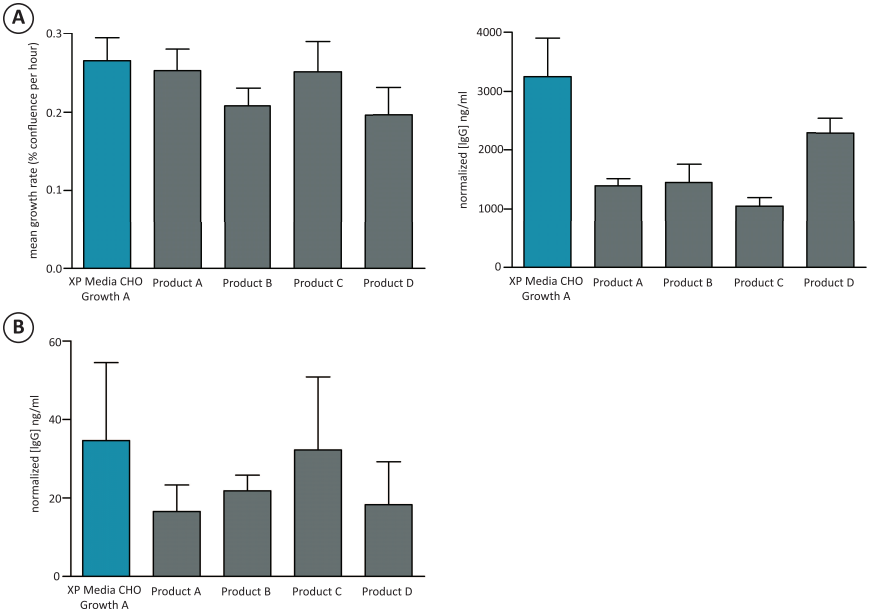

Figure 1-3: Comparison of different chemically defined CHO media on post-pick recovery and productivity of suspension adapted B13-24 cells

Item	Decsription
A	Mean growth rates and antibody productivity (normalized to 100% cell confluence) of B13-24 cell clones 10 days after picking into XP Media CHO Growth A reagent or alternative competitor products
B	Productivity (normalized to viable cell number) of clones (n=3) expanded to shake flask culture

Chapter 2: Materials and Equipment

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Package Components

Table 2-1: Available Reagents

Item	Quantity	Part Number
CloneMedia CHO Growth A with L-Gln, 90 ml	1 bottle	K8810
CloneMedia CHO Growth A with L-Gln, 90 ml	6 bottles	K8800
CloneMedia CHO Growth A without L-Gln, 90 ml	1 bottle	K8840
CloneMedia CHO Growth A without L-Gln, 90 ml	6 bottles	K8830
XP Media CHO Growth A, 1000 ml	1 bottle	K8860

An entire 90 ml bottle of CloneMedia CHO Growth A reagent (K8810) is sufficient 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 CHO Growth A reagent at –20°C. The product is stable for six (6) months from the date of shipment when stored under the recommended conditions.

Before use, thaw CloneMedia CHO Growth A reagent at 2°C to 8°C overnight. Do not shake contents until completely defrosted. When defrosted, CloneMedia CHO Growth A reagent can be stored at 2°C to 8°C for up to one (1) week.

Store XP Media CHO Growth A reagent at 2°C to 8°C. The product is stable for six (6) months from the date of shipment when stored under the recommended conditions.

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

Chapter 3: Assay Protocol

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Adapting Cells to XP Media CHO Growth A Reagent

Although it is not required, Molecular Devices recommends that you adapt cells to XP Media CHO Growth A reagent before plating in CloneMedia CHO Growth A reagent to maximize colony growth. While most cell lines readily adapt to new media, some have difficulty adjusting to the change. Molecular Devices recommends that you use the sequential adaptation protocol that follows.

Note: XP Media CHO Growth A reagent is supplied without L-Glutamine. Molecular Devices recommends supplementing the medium with 4 mM of L-Glutamine (Irvine Scientific #9317), if necessary.

1. Dispense the original chemically defined medium and XP Media CHO Growth A reagent, at a 75% to 25% ratio, into an appropriate culture container.
2. When the cells are in logarithmic growth phase and above 90% viability, passage the cells from the original culture into the blended media from step 1.
3. Incubate the cells for three to four days or until the cells are ready for passage.
4. Maintain the cells in each ratio for at least one passage before proceeding to the next dilution.

   Molecular Devices recommends that you always keep the cells in the previous ratio in case of a sudden crash.
5. Prepare new media with the original medium from step 1 and XP Media CHO Growth A reagent at a 50/50 mix.
6. Subculture the cells into the freshly prepared media from step 5.
7. Repeat steps 3 to 6 with sequential dilution ratios of 25/75 and 100% XP Media CHO Growth A reagent.

   If the cells look unhealthy or the growth rate declines significantly at any step, maintain the cells for another passage in the media ratio of the previous step before subculturing into the next ratio.
8. To ensure optimum performance, maintain the cells in the XP Media CHO Growth A reagent for several passages to allow for complete adaptation.

Preparing CloneMedia CHO Growth A Reagent

Before use:

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

Note: On rare occasions, slight variations in color of CloneMedia CHO Growth A reagent can occur. This does not interfere with cell health and proliferation, or with image quality and analysis.

For 100 ml of semi-solid media:

1. Add any other necessary components, such as selection agents or antibiotics, to the bottle containing 90 ml of CloneMedia CHO Growth A reagent.
2. If necessary, add sterile tissue culture water to raise to a final volume of 100 ml.
   Make sure that you provide enough space for the CloneDetect agent and the volume of cells to be added in subsequent steps.

To mix thoroughly:

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

For fluorescent detection assays:

CloneDetect agent can be added at this stage.

1. Mix CloneDetect agent gently into the prepared media.
2. Protect the bottle from direct light.

For detailed instructions, see the protocol for the CloneDetect agent.

Cell Plating in CloneMedia CHO Growth A Reagent for Serum-Free, Suspension-Adapted CHO Cells

CHO cells growing in semi-solid conditions form discrete, spherical colonies suspended in the semi-solid media, see Figure 1-2: Suspension Adapted B13-24 Cells on page 5.

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 optimize the seeding densities thoroughly.

Seeding densities required to achieve an optimum density of colonies depend on the inherent plating efficiency of the cells used and on 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 CHO cell lines (100 to 2000 cells/ml)
- Fresh transfections (1 x 10^3 to 1 x 10^6 cells/ml)

Note: When plating fresh transfections, the optimum seeding density is highly dependent on the transfection efficiency and the kinetics and efficiency of selection. For the successful selection of transfectants, the selection pressure (for example, antibiotic concentration) applied can require optimization. Cells can also require a period of recovery before seeding in semi-solid media. To ensure clonality, it might be necessary to dissociate cells by gently pipetting the cell suspension up and down. Care must be taken not to damage the cells.

1. Add the appropriate amount of cells to the semi-solid media. Invert the bottle 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.

   Note: It is crucial that appropriately treated culture plastics are used. Plate into **non-TC**-treated culture dishes.

3. Tilt the culture dish gently to ensure even distribution.
4. Place 4 ml of sterile water in the area between wells to ensure that the semi-solid media remains well hydrated.
5. Place the cultures in an incubator at 37°C, 5% CO_2 for 7 to 14 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.

   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.

Cell Plating in CloneMedia CHO Growth A Reagent for Adherent CHO Cells

Molecular Devices ClonePix systems can pick suspended colonies and adherent colonies growing at the bottom of the culture dish, providing that they are discrete from one another.

For adherent CHO cell lines, two different plating protocols have been developed for the growth of colonies in semi-solid media:

- A. Adherent CHO Cells: Plating for Suspended Colonies, see page 11
- B. Adherent CHO Cells: Plating for Adherent Colonies, see page 12

Protocol A is the preferred method, as suspended colonies offer the inherent advantage of being more compact. This lets clones be plated and picked at a higher density with low risk of cross contamination.

However, not all adherent CHO lines are suitable for this approach. Depending on the properties of the CHO line used, cells adhere more or less readily to the bottom of the culture dish and form a layer of adherent cells in the background. For highly adherent CHO lines, protocol B is more suitable.

A. Adherent CHO Cells: Plating for Suspended Colonies

Prepare semi-solid media as described in Preparing CloneMedia CHO Growth A Reagent on page 9 and supplement with a small amount of serum (1% or less, if possible).

To add cells and dispense media, follow the instructions in Cell Plating in CloneMedia CHO Growth A Reagent for Serum-Free, Suspension-Adapted CHO Cells on page 10.

Note: It is crucial that the appropriately treated culture plastics are used. Plate into **non-TC**-treated culture dishes.

B. Adherent CHO Cells: Plating for Adherent Colonies

For the following procedure, plating efficiency is high. Molecular Devices recommends evaluation of a range of seeding densities (25 cells/ml to 50 cells/ml).

1. Plate the cells into **TC-treated** culture dishes in liquid growth media supplemented with serum.
   - In a PetriWell-6 Plate, plate the cells in 2 ml per well.
   - In an Omnitray, plate the cells in 18 ml per tray.
2. Place the plated cells in an incubator at 37˚C, 5% CO_2 overnight and allow the cells to adhere.
3. Verify good cell adherence using a light microscope.
4. Prepare CloneMedia CHO Growth A reagent as described in Preparing CloneMedia CHO Growth A Reagent on page 9, and supplement with the same amount of serum as used in routine liquid culture.
5. Carefully aspirate the liquid media from the wells.
6. Gently overlay the cells with CloneMedia CHO Growth A reagent immediately using a pipette (for example, 10 ml) or by pouring.
   - In a PetriWell-6 Plate, dispense approximately 2 ml per well.
   - In an Omnitray, dispense approximately 18 ml per tray.
7. Tilt the culture dish gently to ensure even distribution.
8. Place 4 ml of sterile water in the area between the wells to ensure that the semi-solid media remains well hydrated.
9. Place the cultures in an incubator at 37°C, 5% CO_2 for 2 to 10 days to allow the colonies to grow.

Expansion of Clones with XP Media CHO Growth A Reagent

Following automated picking of clones from CloneMedia CHO Growth A reagent, XP Media CHO Growth A reagent provides a liquid media optimized for supporting recovery, growth, and expansion of the selected clones.

Note: Supplement XP Media CHO Growth A reagent with L-Glutamine, selection agent, or serum where required.

1. Pick the cell colonies, using a ClonePix 2 system, into 96-well microplates containing 150 µl to 200 µl of XP Media CHO Growth A reagent.
2. Let the picked clones reach high (>80%) confluence.

   If necessary, the cells can be fed with fresh media after ten (10) days.
3. Transfer the clones from the 96-well microplate to a 12-well cell culture plate containing 1 ml of XP Media CHO Growth A reagent and let the cells reach high (>80%) confluence.
4. Passage the cells to 6-well cell culture plates containing 2 ml to 3 ml of XP Media CHO Growth A reagent and let the cells reach high (>80%) confluence.
5. Passage the cells to shake flask culture.

   The optimum cell density is cell-line dependent. Molecular Devices recommends seeding at 5 x 10^4 to 2 x 10^5 viable cells/ml in XP Media CHO Growth A reagent.
6. Maintain the cells by passaging every 3 to 4 days.

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 , 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.

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