Table of Contents

3  ClonaCell™ Products for Cell Line Development
4  Semi-Solid Cloning for Cell Line Development
5  Semi-Solid Cloning Delivers a High Probability of Monoclonality
6  Semi-Solid Media for Semi-Solid Cloning of CHO Cells
7  Liquid Media for Adaptation, Expansion and Cloning of CHO Cells
8  Products for Hybridoma Generation
10 Automated, High-Throughput Cell Line Development
    with ClonaCell™ EasyPick

STEMCELL Technologies Inc., a leader in specialty cell culture media, cell separation products and cell based reagents, is a biotechnology company based in Vancouver, Canada. Driven by science, STEMCELL Technologies delivers over 1500 products to more than 70 countries worldwide. To learn more about how STEMCELL Technologies helps make research work, visit www.stemcell.com.
The ClonaCell™ product line from STEMCELL Technologies offers innovative solutions for cloning mammalian cell lines and hybridomas.

Save time and resources during the development of mammalian cell lines with ClonaCell™ specialized cloning media. Semi-solid cloning with ClonaCell™ methylcellulose-based media is a superior cloning method that allows you to overcome the challenges of traditional limiting dilution cloning for developing mammalian cell lines. ClonaCell™ media streamline your cell line development workflow by achieving a high probability of monoclonality and high clonal diversity in a single cloning step.

STEMCELL Technologies provides ClonaCell™ products for cloning CHO cells and hybridomas, including animal component-free, chemically defined and pre-screened serum-containing formulations. With uncompromising quality, innovative cloning techniques and technology for automation of cell line development, ClonaCell™ products help you to take the smart way to the right clone.

To find out more about ClonaCell™ products, please visit www.clonacell.com.
Semi-solid cloning saves time and materials for cell line development. During traditional limiting dilution cloning, individual wells may contain more than one clone; therefore, subcloning is essential to obtain a monoclonal culture. During semi-solid cloning, however, individual cells are immobilized within the viscous medium and grow into discrete, monoclonal colonies. Picking these colonies enables isolation of diverse clones with a high probability of monoclonality in a single cloning step.

**Benefits of Semi-Solid Cloning with ClonaCell™:**
- High Probability of Monoclonality
- No Resources Wasted on Low Cloning Efficiency
- No More Limiting Dilution
Semi-Solid Cloning Delivers a High Probability of Monoclonality

Semi-solid cloning with ClonaCell™ media can achieve the same probability of monoclonality in fewer rounds of cloning and using fewer resources than limiting dilution cloning (LDC), leading to faster cell line development timelines.

In LDC, low seeding densities are used to increase the probability of isolating individual cells in unique wells. Statistically, however, some wells will be seeded with more than one cell, even at very low seeding densities. Multiple rounds of LDC are therefore required to achieve a high probability of monoclonality.

Semi-solid cloning overcomes these limitations by allowing individual cells to grow into discrete colonies, making it easy to isolate cultures with a high probability of monoclonality.

Estimated Probability of Monoclonality for LDC and Semi-Solid Cloning Experiments*

<table>
<thead>
<tr>
<th>LIMITING DILUTION CLONING</th>
<th>PROBABILITY OF MONOCOLONALITY (%)**</th>
<th>SEMI-SOLID CLONING</th>
<th>PROBABILITY OF MONOCOLONALITY (%)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty Wells (%)</td>
<td>Initial Round of Cloning</td>
<td>Total Colonies per 10 cm Dish</td>
<td>Initial Round of Cloning</td>
</tr>
<tr>
<td></td>
<td>1st Subcloning Round</td>
<td></td>
<td>1st Subcloning Round</td>
</tr>
<tr>
<td>30</td>
<td>30.5</td>
<td>160</td>
<td>96.4</td>
</tr>
<tr>
<td>35</td>
<td>35.6</td>
<td>150</td>
<td>96.6</td>
</tr>
<tr>
<td>40</td>
<td>40.6</td>
<td>140</td>
<td>96.9</td>
</tr>
<tr>
<td>45</td>
<td>45.6</td>
<td>130</td>
<td>97.1</td>
</tr>
<tr>
<td>50</td>
<td>50.6</td>
<td>120</td>
<td>97.3</td>
</tr>
<tr>
<td>55</td>
<td>55.6</td>
<td>110</td>
<td>97.5</td>
</tr>
<tr>
<td>60</td>
<td>60.6</td>
<td>100</td>
<td>97.8</td>
</tr>
<tr>
<td>65</td>
<td>65.6</td>
<td>90</td>
<td>98.0</td>
</tr>
<tr>
<td>70</td>
<td>70.5</td>
<td>80</td>
<td>98.2</td>
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<tr>
<td>75</td>
<td>75.5</td>
<td>70</td>
<td>98.4</td>
</tr>
<tr>
<td>80</td>
<td>80.4</td>
<td>60</td>
<td>98.7</td>
</tr>
<tr>
<td>85</td>
<td>85.3</td>
<td>50</td>
<td>98.9</td>
</tr>
</tbody>
</table>

*Calculations are based on R. Staszewski, 1984 and the webinar “A Smarter Way to Clone”2
**Based on an LDC experiment where 5% of cultures obtained are carried forward for subcloning or downstream experiments
***Based on a semi-solid cloning experiment where the average colony diameter is 0.75 mm, all colonies are picked and any colony which touches or overlaps with another colony gives rise to a polyclonal culture

Learn why rare, high-producing cultures isolated with LDC have a lower probability of monoclonality than a random culture in the same cloning experiment by watching our on-demand webinar.

2. www.stemcell.com/smarter_way_to_clone

WEBINAR
A Smarter Way to Clone: What You Don’t Know About Limiting Dilution Can Hurt Your Probability of Monoclonality
www.stemcell.com/smarter_way_to_clone
Semi-Solid Media for Semi-Solid Cloning of CHO Cells

Chemically defined, animal component-free and serum-containing media formulations are available for semi-solid cloning of CHO cells.

Chemically Defined Media
Chemically defined media can be used when a protein-free and animal component-free formulation is required.

PRODUCT: ClonaCell™-CHO CD
CATALOG #: 03815  90 mL

ClonaCell™-CHO CD is a methylcellulose-based semi-solid medium recommended for the selection and cloning of CHO cells. The medium is chemically defined, protein-free, animal component-free and does not contain L-glutamine or selective agents. The medium is suitable for dihydrofolate reductase (DHFR) and glutamine synthase (GS) selection.

PRODUCT: ClonaCell™-CHO ACF
CATALOG #: 03816  90 mL

ClonaCell™-CHO ACF is a methylcellulose-based semi-solid medium recommended for the selection and cloning of CHO cells. The medium contains recombinant proteins, is animal component-free and does not contain L-glutamine or selective agents. The medium is suitable for DHFR and GS selection.

PRODUCT: ClonaCell™ FLEX
CATALOG #: 03818  45 mL

ClonaCell™ FLEX is a base methylcellulose medium recommended for the selection and cloning of mammalian cells. The medium is chemically defined, protein-free, animal component-free and does not contain L-glutamine or selective agents. The FLEX format enables greater flexibility as it allows the user to add an equal volume of their own 2X liquid medium. The medium is suitable for DHFR and GS selection.

Animal Component-Free Medium
Animal component-free media can be used for applications where a serum-free medium is required and the presence of recombinant proteins is acceptable.

PRODUCT: ClonaCell™-CHO ACF
CATALOG #: 03816  90 mL

ClonaCell™-CHO ACF is a methylcellulose-based semi-solid medium recommended for the selection and cloning of CHO cells. The medium contains recombinant proteins, is animal component-free and does not contain L-glutamine or selective agents. The medium is suitable for DHFR and GS selection.

Serum-Containing Medium
Serum-containing medium is a robust option for cloning and selection of CHO cells and other cell lines. It can be used for applications where the presence of serum is acceptable.

PRODUCT: ClonaCell™-TCS
CATALOG #: 03814  80 mL

ClonaCell™-TCS is a semi-solid medium recommended for the selection and cloning of cell lines including CHO, BHK-21 and HEK-293. The medium contains methylcellulose, pre-selected fetal bovine serum and BSA, and does not contain selective agents.
Liquid Media for Adaptation, Expansion and Cloning of CHO Cells

Chemically defined and animal component-free liquid media that have been optimized for use with our semi-solid media are available for the adaptation, expansion and cloning of CHO cells.

Chemically Defined Medium

**PRODUCT:** ClonaCell™-CHO CD Liquid  
**CATALOG #:** 03817 500 mL

ClonaCell™-CHO CD Liquid is a liquid medium recommended for adaptation or expansion of CHO cells. The medium is chemically defined, protein-free, animal component-free and does not contain L-glutamine or selective agents. The medium is suitable for dihydrofolate reductase (DHFR) and glutamine synthase (GS) selection.

Animal Component-Free Medium Supplement

**PRODUCT:** ClonaCell™-CHO ACF Supplement  
**CATALOG #:** 03820 2.5 mL

ClonaCell™-CHO ACF Supplement is an animal component-free media supplement for the culture of CHO cells. The 40X supplement concentrate may be used with your own cell culture medium to significantly increase the cloning efficiency of CHO cells during single-cell cloning for cell line development. It may be used to supplement either liquid or semi-solid protein-free medium.

**VIDEO**  
ClonaCell™-CHO Procedure  
www.stemcell.com/Video-ClonaCell-CHO-Procedure

Chemically defined and animal component-free media offer the following advantages:

- Decreased risk of introducing adventitious agents
- Increased process consistency
ClonaCell™-HY products offer a complete solution for the development of monoclonal antibodies, from cell fusion to the selection and growth of hybridomas.

**ClonaCell™-HY Kit (Components Can Be Purchased Separately)**

<table>
<thead>
<tr>
<th>CLONACELL™-HY PRODUCT</th>
<th>VOLUME</th>
<th>DESCRIPTION</th>
<th>CONTAINS</th>
<th>CATALOG #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium A (Pre-Fusion)</td>
<td>500 mL</td>
<td>Myeloma growth medium</td>
<td>Pre-selected serum, gentamycin and supplements</td>
<td>03801</td>
</tr>
<tr>
<td>Medium B (Fusion)</td>
<td>500 mL</td>
<td>Medium used to wash cells prior to cell fusion and for use during fusion</td>
<td>Gentamycin</td>
<td>03802</td>
</tr>
<tr>
<td>Medium C (Recovery)</td>
<td>100 mL</td>
<td>Fusion recovery medium to promote hybridoma viability</td>
<td>Pre-selected serum, gentamycin and supplements</td>
<td>03803</td>
</tr>
<tr>
<td>Medium D (Selection &amp; Cloning)</td>
<td>90 mL</td>
<td>Semi-solid HAT hybridoma selection medium</td>
<td>Methylcellulose, pre-selected serum, HAT, gentamycin and supplements</td>
<td>03804</td>
</tr>
<tr>
<td>Medium E (Growth)</td>
<td>500 mL</td>
<td>Hybridoma growth medium</td>
<td>Pre-selected serum, HT, gentamycin and supplements</td>
<td>03805</td>
</tr>
<tr>
<td>PEG</td>
<td>1.5 mL</td>
<td>Solution for cell fusion</td>
<td>50% PEG</td>
<td>03806</td>
</tr>
</tbody>
</table>

HAT = hypoxanthine-aminopterin-thymidine; HT = hypoxanthine-thymidine; PEG = polyethylene glycol

With the ClonaCell™-HY method, a methylcellulose-based semi-solid selection medium is used to combine hypoxanthine-aminopterin-thymidine (HAT) selection and cloning of hybridomas in one step. Single cell-derived hybridomas form discrete, monoclonal colonies in the semi-solid medium. The hybridomas can be easily picked, screened and expanded to produce the desired antibody. The kit contains one bottle of each: ClonaCell™-HY Medium A, B, C, D, E and ClonaCell™-HY Polyethylene Glycol (PEG). Kit components can be purchased separately.
### ClonaCell™ FLEX

**PRODUCT:** ClonaCell™ FLEX  
**CATALOG #:** 03818  
**Volume:** 45 mL

ClonaCell™ FLEX is a base methylcellulose medium recommended for the selection and cloning of mammalian cells. The medium is chemically defined, protein-free, animal component-free and does not contain L-glutamine or selective agents. The FLEX format enables greater flexibility as it allows the user to add an equal volume of their own 2X liquid medium.

### ClonaCell™-HY Medium D Without HAT

**PRODUCT:** ClonaCell™-HY Medium D Without HAT  
**CATALOG #:** 03810  
**Volume:** 90 mL

ClonaCell™-HY Medium D Without HAT is a methylcellulose-based semi-solid medium that does not contain any selection reagents. This medium is suitable for cloning hybridoma and myeloma cells. Appropriate selection reagents may be added to select for hybridoma fusion products or stable myeloma transfectants.
Automated, High-Throughput Cell Line Development with

ClonaCell™ EasyPick

The ClonaCell™ EasyPick is a platform for automated cell line development that merges semi-solid cloning using ClonaCell™ media with HAMILTON’s 30 years of experience in liquid-handling robotics. The ClonaCell™ EasyPick provides the quality, speed and throughput required for industrial-scale development of mammalian cell lines.

VIDEO
The ClonaCell™ EasyPick
www.stemcell.com/EasyPickVid
The Smart Way To The Right Clone

Speed and Throughput
The ClonaCell™ EasyPick platform is the world’s fastest system for automated mammalian colony isolation. With the ability to perform >200 clone isolations per hour,* the ClonaCell™ EasyPick not only improves process consistency, but offers significant time savings over manual colony picking or other cloning methods.

*Based on averaged values that include image acquisition time, image analysis time, pre-wet time and plate/lid movement time.

Process Safety
The ClonaCell™ EasyPick system uses disposable tips for the picking process. This minimizes the risk of contamination. Controlled, smooth tip pick-up and disposal by HAMILTON’s Compressed O-Ring Expansion (CO-RE) technology further decreases the risk of aerosol formation.

Multi-Parameter Selection
HAMILTON’s proprietary EasyPick software uses multiple parameters (including colony size, circularity and proximity to neighboring colonies) that are custom-defined and weighted to meet the requirements of each user. The custom-programmed software ensures process consistency and reliability during the development of mammalian cell lines.

Quality and Reliability
Each module is tested with criteria that are higher than those required for the instrument as a whole. Pipetting channels are tested for 10 million cycles during design and core parts are manufactured by HAMILTON to the highest tolerances.

Scalability and Versatility
The ClonaCell™ EasyPick platform is designed for modularity and scalability. The ClonaCell™ EasyPick can be configured to allow for the automation of upstream or downstream processes, such as cell culture or ELISA screening, through the integration of additional modules. The exchange of modular carriers for various applications enables versatility on the same platform. This allows for more efficient resource management to meet the changing demands of drug discovery.