Get to your high-value clones faster with a complete hybridoma media solution

BENEFITS

- **Complete solution from hybridoma fusion to expansion**
- **Simultaneous cloning and selection of hybridomas**
- **Optimized for high production and fast growth**

Introduction

During the last decade, the rising demand for monoclonal antibodies (mAbs) has led to a boom in the introduction of biotherapeutic proteins. As the interest in this research area continues to increase, the need to bring down cell line development costs and shorten time to market is more critical than ever.

We offer a comprehensive, high-throughput colony picking and screening technology with optimally-formulated media to maximize productivity and minimize hands-on time for antibody discovery. The ClonePix™ 2 System, a mammalian colony picker, allows for fluorescent screening and picking of high, antibody-producing hybridomas when a fluorescently-labeled antibody, such as FITC-labeled CloneDetect Antibody, is added to the media. CloneSelect™ Imager, a label-free imaging system, allows for objective, quantitative assessment of cell growth and monoclonality verification, ensuring that only monoclonal antibodies are selected for downstream studies. The CloneMedia and XP Media suite of hybridoma media offers a complete solution for generating, culturing, cloning, screening, and scaling up of hybridomas on our systems and are described in further detail.

Workflow for antibody discovery in hybridomas

The traditional approach to generating mAb-producing cells (i.e. hybridomas) is to fuse myeloma cells with desired antibody-producing splenocytes (e.g. B cells). These B cells are typically sourced from animals, usually mice. Following cell fusion, large numbers of clones are screened and selected on the basis of antigen specificity and immunoglobulin class. Once candidate cell line clones are identified, each “hit” is re-confirmed, validated, and characterized using a variety of downstream functional assays. Once completed, the clones are expanded or scaled up where additional downstream bioprocesses occur. Here we provide a general overview of the hybridoma workflow (Figure 1) along with data supporting the use of our XP Media and CloneMedia suite of hybridoma media products.

MOLECULAR DEVICES

Accelerate your hybridoma cell line development with a complete set of platforms and culture media

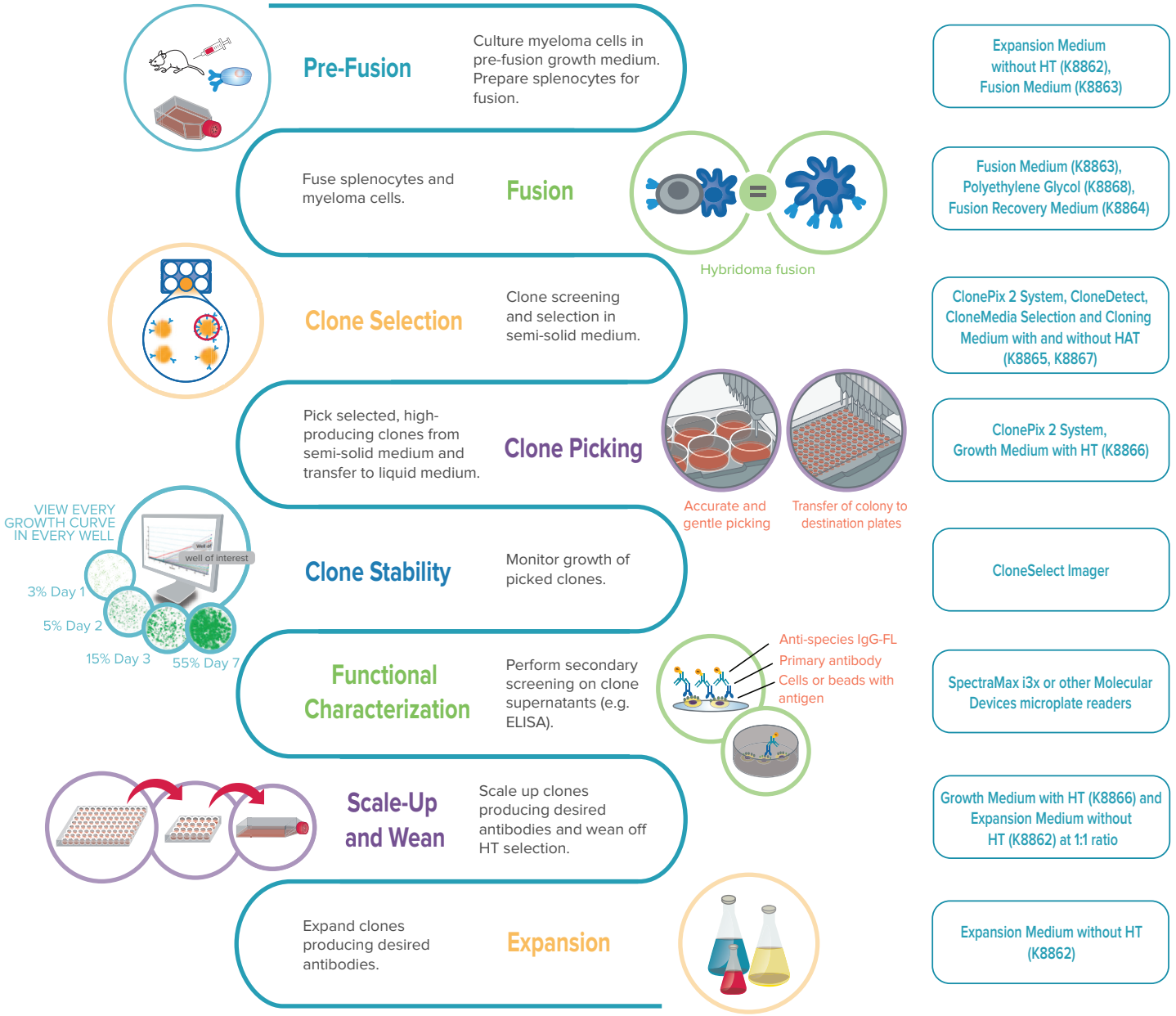


Figure 1. Hybridoma workflow for antibody discovery.

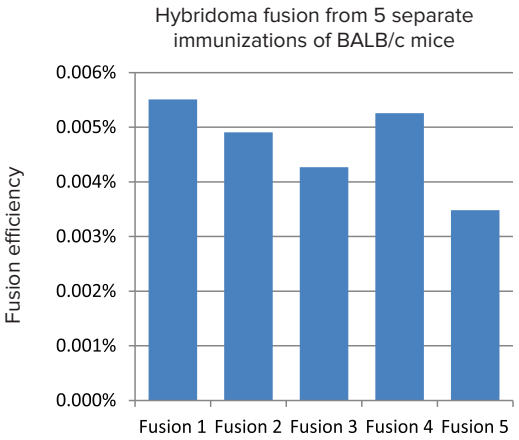


Figure 2. Fusion efficiency of hybridomas. The efficiency of PEG fusion of hybridomas was calculated by dividing the number of colonies recorded in semi-solid media by the splenocyte count prior to fusion. Consistent fusion efficiencies were observed across 5 independent experiments and are comparable with hybridoma fusion efficiencies from other techniques.

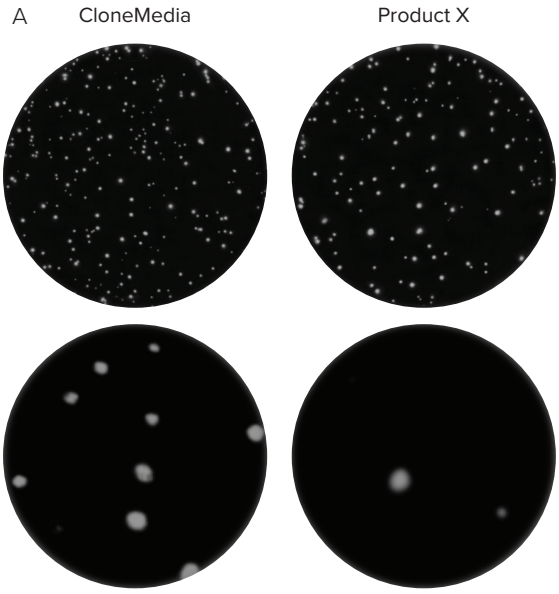


Figure 3. Hybridomas cultured in different semi-solid media. **(A)** Hybridomas were plated in CloneMedia semi-solid media from Molecular Devices and in semi-solid media from Competitor X. A greater number of clones were able to grow in CloneMedia when compared to Product X as shown in the white light images taken of colonies on the ClonePix 2. **(B)** Hybridoma viability was calculated by dividing the number of colonies detected by the initial seeding density. Hybridomas were more viable in CloneMedia than in Product X. **(C)** This was further confirmed by comparing the average colony size in both media.

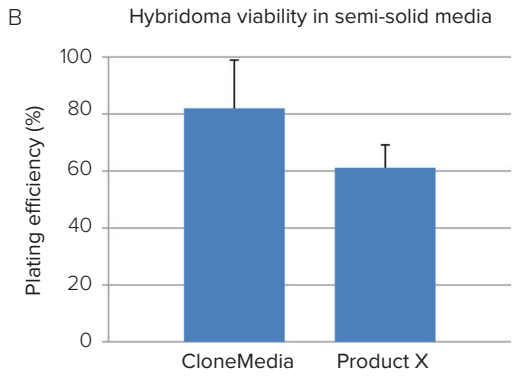
Hybridoma generation by PEG fusion

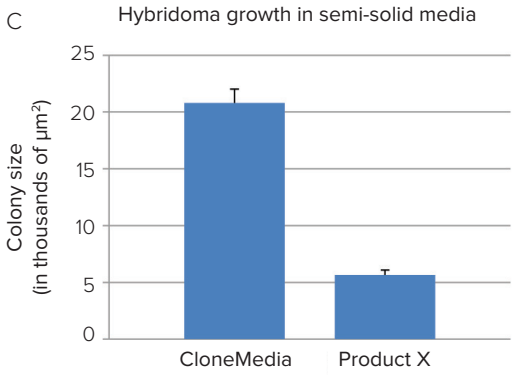
Myeloma cells grown in XP Media Pre-Fusion Myeloma Growth Medium and Hybridoma Expansion Medium (without HT) were fused with splenocytes using the Hybridoma Polyethylene Glycol (PEG) for Cell Fusion. Prior to fusion, it is important to wash the splenocytes in serum-free XP Media Hybridoma Fusion Medium, otherwise fusion efficiency is greatly diminished. Hybridomas were then cultured in XP Media Hybridoma Fusion Recovery Medium for 24 hours before plating in semi-solid media. Figure 2 illustrates the fusion efficiencies observed following 5 independent hybridoma generation experiments.

Superior viability and growth of hybridomas in semi-solid media

CloneMedia Hybridoma Semi-Solid Selection and Cloning Medium (with HAT) is a semi-solid methylcellulose-based medium containing supplements to promote the growth of single-cell hybridomas into colonies in the presence of the selection agents hypoxanthine, aminopterin, and thymidine (HAT) (Figure 3).

Colonies grown in CloneMedia are larger in size and more densely packed, indicating better growth and viability, respectively, than Product X.





Significant time savings with high-throughput fluorescence screening

The addition of a fluorescently-labeled antibody such as FITC-labeled CloneDetect to CloneMedia hybridoma semi-solid media allows for fluorescent screening of antigen specificity or total IgG production. The ClonePix 2 System fluorescently images antibody secretion of hybridomas (Figure 4) and subsequently ranks and picks colonies based on the FITC intensity. This allows the ClonePix 2 System to select only the highest expressors amongst thousands of colonies, which greatly reduces the mAb workload by decreasing the number of sub-cloning steps required.

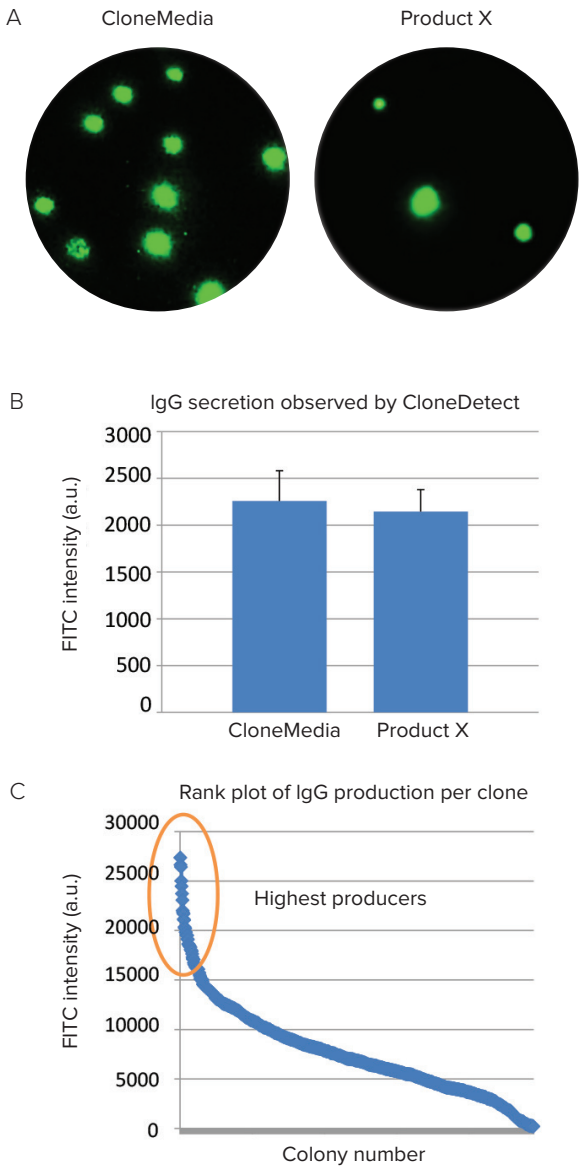


Figure 4. Selection and picking of highest producing colonies. **(A, B)** Fluorescence images of hybridomas plated in semi-solid media with CloneDetect-FITC were captured with the ClonePix 2 System to assess total IgG production. **(C)** Clones were ranked based on their FITC intensity and subsequently picked into a 96-well plate for further characterization.

Optimal growth and antibody production of hybidomas in liquid media

The highest-producing hybridoma colonies picked by the ClonePix 2 System can then be grown in cell culture plates containing XP Media Hybridoma Growth Medium. Hybridomas cultured in our XP Media and CloneMedia have superior growth and antibody productivity compared to hybridoma offerings offered by another vendor as shown in Figure 5. The development and proliferation of high-secreting clones enable more efficient workflows by increasing the number and quality of candidates moved on to the scale-up stage.

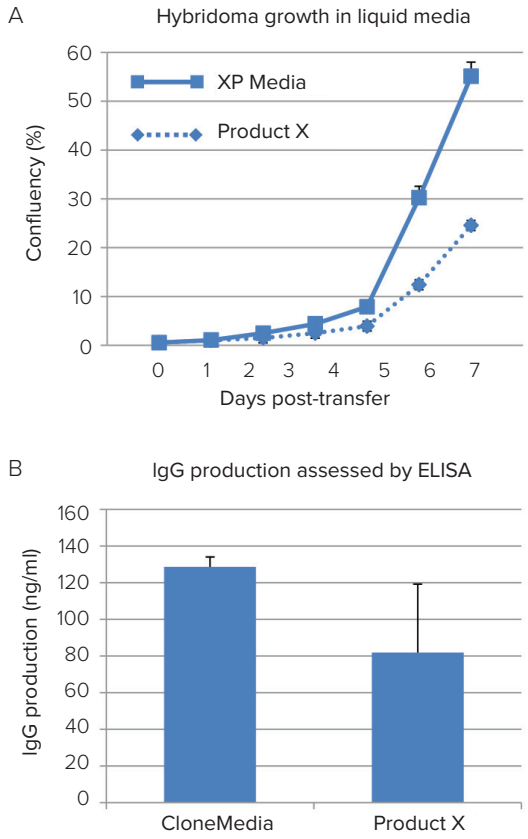


Figure 5. Growth and antibody production of hybridomas post-selection. 96 top producing clones were picked from semi-solid media into a 96-well plate and their growth was monitored over 7 days. **(A)** Hybridomas grown in XP Media grew 2X as fast as Product X but did not suffer from a reduction in antibody production due to enhanced growth, as shown in **(B)**.

Summary

We provides a fast, simple and comprehensive solution for hybridoma screening. When used together, XP Media, CloneMedia, CloneDetect, CloneSelect Imager, and ClonePix 2 System enable researchers to more efficiently discover novel monoclonal antibodies, shortening the time to market.

Item	Quantity	Part number
XP Media and CloneMedia Complete Kit for Mouse Hybridoma Generation	1 each of the following part numbers: K8862, K8863, K8864, K8865, K8866, K8868	K8861

Table 1. Available kits.

Item	Quantity	Part number
XP Media Pre-Fusion Myeloma Growth Medium and Hybridoma Expansion Medium (without HT)	500 mL	K8862
XP Media Hybridoma Fusion Medium	500 mL	K8863
XP Media Hybridoma Fusion Recovery Medium	100 mL	K8864
CloneMedia Hybridoma Semi-Solid Selection and Cloning Medium (with HAT)	90 mL	K8865
XP Media Hybridoma Growth Medium (with HT)	500 mL	K8866
CloneMedia Hybridoma Semi-Solid Selection and Cloning Medium (without HAT)	90 mL	K8867
Hybridoma Polyethylene Glycol (PEG) for Cell Fusion	1.5 mL	K8868

Table 2. Available media.

To Order

Phone: +1-800-635-5577, select #2
Email: om@moleculardevices.com

Contact Tech Support

Phone: +1-800-635-5577, select #3
Web: www.moleculardevices.com
Email: info@moldev.com
Check our website for a current listing of worldwide distributors.

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