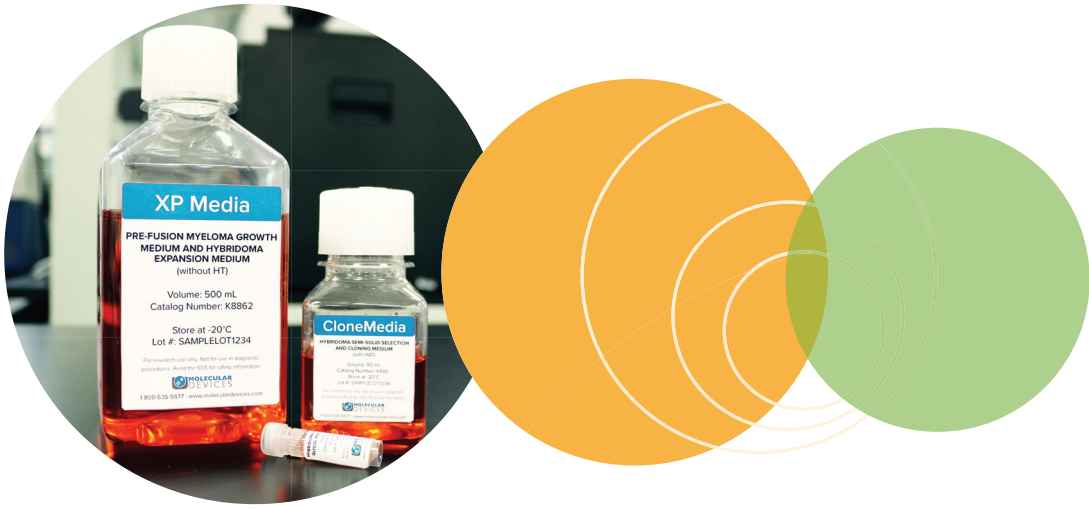



FREQUENTLY ASKED QUESTIONS

# XP Media and CloneMedia for Mouse Hybridoma Generation

## What is the formulation of the medium?

Although the full formulation is proprietary, we can disclose some key components. The semi-solid medium is a methylcellulose-based medium containing serum, supplements, and selective reagents hypoxanthine, aminopterin, and thymidine (HAT). P/N K8866 contains only HT and P/N K8867 does not contain HAT. The liquid medium contains Dulbecco's Modified Eagle's Medium (DMEM), serum (except P/N K8863), gentamycin, and supplements.

## Does the semi-solid medium contain HAT (hypoxanthine, aminopterin, thymidine)?

Yes, P/N K8865 contains HAT. We also offer semi-solid medium without HAT, P/N K8867.

## Does the medium contain phenol red?

Yes.

## After thawing the medium, do I need to use it all at once?

No. The media can be stored at 2 - 8°C for varying lengths of time without loss of performance. P/N K8862 can be stored at 2 -8°C for up to 1 month. P/N K8863 can be stored at 2 - 8°C for up to 4 months. P/N K8864, K8865, and K8866 can be stored at 2 - 8°C for up to 14 days.

## Can I refreeze the medium after thawing?

The medium should not be subjected to multiple freeze-thaw cycles. If needed, the medium can be thawed, aliquoted into appropriate volumes then stored at -20°C until expiration date indicated on the original label.

## Why do I get more cells when I select my fusion in liquid medium rather than in methylcellulose-based semi-solid medium?

Cells grown in liquid medium cover a larger area of a well than cells grown in semi-solid medium, making them appear to grow more rapidly. However, this is just an optical illusion. Rapidly dividing cells are able to freely move through liquid media, but rapidly dividing cells grown in semi-solid media are confined to a much smaller area. Thus, cell numbers should be similar if plated under similar conditions.

## How do I thaw CloneMedia hybridoma methylcellulose-based semi-solid medium?

CloneMedia hybridoma methycellulose-based semi-solid medium should be thawed overnight at 2-8°C or room temperature to avoid rapid warming of the medium, which can alter the chemical structure of methycellulose. DO NOT place in a warmed water bath.

## How do I measure and dispense methylcellulose semi-solid medium?

The methylcellulose semi-solid medium is highly viscous, which can make measuring and dispensing it more challenging than liquid media. Traditional methods of aspirating and dispensing (such as pipettes) should not be used as the medium will stick to the pipette, causing inaccuracies. We recommend using a syringe with a 16-gauge needle attached (blunt-end needles are recommended for safety purposes), but take care to aspirate and dispense slowly in order to avoid the generation of bubbles.

MOLECULAR DEVICES

## My CloneMedia hybridoma methylcellulose semi-solid medium appears runny. Why does this happen?

Semi-solid media that is too "runny" will result in streaky or runny colonies following cell plating. Keep this in mind when evaluating the viscosity of the semi-solid media. If streaky or runny colonies are observed, look to the following actions that may result in altered viscosity as a guide to help troubleshoot.

- Inefficient mixing
- Too much liquid medium added to the semi-solid medium
- Improper thawing or warming of semi-solid medium
- Multiple freeze/thaw cycles
- Excessive condensation on the inside of the culture plate
- Frequent and/or abrupt movement of plates. Disturbing the dishes before Day 10 will break apart small forming colonies and cause them to appear hazy or runny.

If appropriate, you can prepare an extra plate as an "observation plate" to monitor colony growth during the incubation period. Allow the observation plate to incubate undisturbed for at least 4 days before viewing or imaging to allow the single cells to start to divide and grow. Beyond 4 days, you can observe this plate daily until a suitable colony size is reached.

## What is the optimal number of colonies per plate?

The optimal number of cells to plate per well can vary depending on the cell type, cell culture, passage number, etc. We suggest plating at different densities to determine optimal seeding densities. If utilizing a Molecular Devices ClonePix™ 2 System, we recommend a final density of approximately 200-400 colonies per well on a standard 6-well plate. If manually picking with a handheld pipettor, we recommend a final density of approximately 5-15 colonies per well on a standard 6-well plate.

## There are still bubbles in the media after I plate my cells. Do I need to disrupt the bubbles?

A small number of large bubbles (>1 mm) may occur due to the viscosity of the medium. Bubbles interfere with the ability of the software to image and identify properly growing clones. Thus, with 1-2 bubbles per well, you can expect to lose up to 5% of colonies. We suggest using a sterile pipette tip to remove large bubbles. Small bubbles (<1 mm) that are difficult to see by eye should not pose any concern as they will disperse during the incubation period.

## Do I ever need to re-clone cultures grown with CloneMedia hybridoma semi-solid medium?

The advantage of using semi-solid media is that the growth of single cells can be restricted in space, resulting in colonies that are monoclonal. This minimizes the need to re-clone cultures grown in CloneMedia, reducing the amount of time required to identify the highest producers. However, re-cloning is recommended if the number of colonies in the original plate was very high or as a best practice to help ensure monoclonality.

## Once I pick the colonies and grow the cells in plates, will the residual methylcellulose interfere with characterization? For example, will I have problems doing an ELISA?

There may be small amounts of methylcellulose transferred to the 96-well plate during the picking procedure. However, this should not interfere with most assays including ELISA.

## How important is the incubator humidity when culturing in methylcellulose-based medium?

It is very important to maintain humidity when using semi-solid medium. If the medium dries, you can expect very little colony growth. We recommend filling the spaces between the wells with sterile water to further maintain humidity across the plate.

## Can I use cultureware other than 6-well plates or omnitrays?

We have found that the surface area of these dishes allows for easy colony picking, but other types and sizes can be used. If using the ClonePix 2 System to pick colonies, refer to the system manual to ensure the plate is compatible with the system. It is important to use non-coated dishes to prevent cells from sticking to the bottom of the plate and obscuring the colonies. The volume of medium plated should be adjusted to reflect the surface area of the plate being used.

## Is the serum in XP Media and CloneMedia heat-inactivated?

Yes, all sera used in XP Media and CloneMedia for Mouse Hybridoma Generation is heat-inactivated.

## Are there antibiotics in XP Media and CloneMedia for mouse hybridoma generation media?

Yes, gentamycin is included and is non-toxic to most mammalian cells in culture.

## Why do I have to put my fused cells into liquid medium overnight? Why can't I plate directly into semi-solid medium?

In order for hybridomas to survive the selection pressures of aminopterin, they must express HPRT (the enzyme required for the secondary DNA synthesis pathway). We recommend letting cells incubate up to 24 hours in liquid medium so that the fused cells have time to express the HPRT enzyme. In addition, recently fused cells are sensitive to their environment, so minimizing perturbations during this 24 hour period will improve their survival.

## What myeloma and mouse strains should I use?

**Myeloma:** SP2/0 and P3X63Ag8.683 are two commonly used cell lines for hybridoma generation and are available from ATCC. We recommend verifying that the source of the myeloma cells is negative for mycoplasma as this can significantly reduce the efficiency of hybridoma formation.

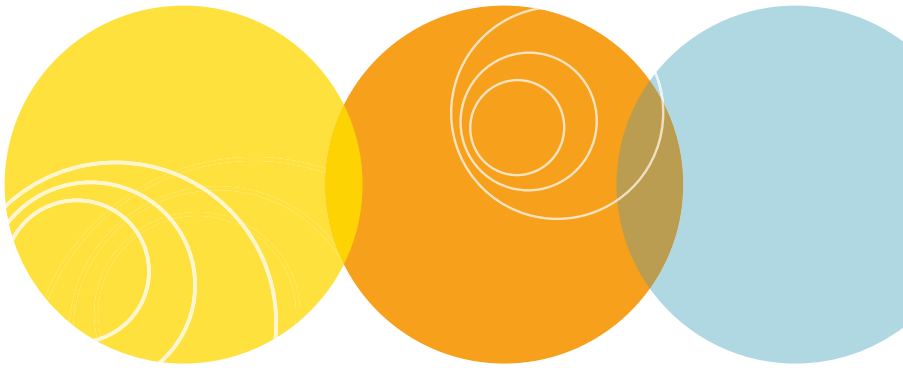
**Mouse:** We recommend using BALB/c splenocytes and parental myeloma cells of BALB/c for hybridoma generation because they are well characterized, highly immune-reactive, and myeloma cells are available from the same genetic strain. Other mouse strains are also compatible, but further testing may be required for optimization of the hybridoma fusions.

### Can I grow human/rat/T cell hybridomas in XP Media and CloneMedia for mouse hybridoma generation?

We have not tested XP Media or CloneMedia for mouse hybridoma generation of human, rat, or T cell hybridomas. We expect the media to work well, but further testing would be needed. At minimum, the researcher would need to ensure that the cell lines used in the fusion are sensitive to HAT selection and grow well in methylcellulose-based medium. If you conduct this test, we would be interested in your feedback.

### Why are only a few colonies growing in my CloneMedia Hybridoma Semi-Solid Selection and Cloning Medium?

Low numbers of colonies is generally a result of low fusion efficiency, which can have many causes. The fusion efficiency can be affected by the presence of serum during fusion, the presence of mycoplasma, low viability of cells, overexposure to polyethylene glycol, or slow-growing myeloma cells prior to fusion.



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