For life science research only.
Not for use in diagnostic procedures.

Hybridoma Fusion and Cloning Supplement (HFCS)

Version: 20
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Media supplement

Cat. No. 11 363 735 001	10 ml 50x conc.

Store product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Hybridoma Fusion and Cloning Supplement (HFCS), 50x conc.	▪ Solution, containing albumin, insulin, transferrin, cytokines, a cholesterol source, other defined organic and inorganic compounds, and 0.5% FCS (fetal calf serum), pH 7.4. ▪ Filtered through 0.2 µm pore size membrane.	1 bottle, 10 ml

1.2. Storage and Stability

Storage Conditions (Product)

The product is shipped on dry ice.
When stored at −15 to −25°C, the product is stable through the expiration date printed on the label.

Vial / Bottle	Label	Storage
1	Hybridoma Fusion and Cloning Supplement (HFCS), 50x conc.	Store in aliquots at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***

1.3. Additional Equipment and Reagent required

For fusion of cells

- Culture medium: Basal medium, such as RPMI 1640 without additional supplements
- Polyethylene glycol, such as PEG 1500*
- Fetal calf serum for the resuspension of the cells
- DMSO

For selection to avoid the use of feeder cells

Serum concentration	Selection media formulation
High-serum	▪ Any basal medium, such as RPMI 1640 ▪ 10% FCS (v/v) ▪ 1x HAT Medium Supplement ▪ 10% BM Condimed[1] H1* (v/v) ▪ 2 mM L-glutamine ▪ 24 µM β-mercaptoethanol
Low-serum	▪ Any basal medium, such as RPMI 1640 ▪ 1x Hybridoma Fusion and Cloning Supplement (HFCS) ▪ 1x HAT Medium Supplement ▪ 2 mM L-glutamine ▪ 24 µM β-mercaptoethanol
Serum-free	▪ Any basal medium, such as RPMI 1640 ▪ 1x Nutridoma-CS[2]* ▪ 1x HAT Medium Supplement ▪ 2 mM L-glutamine ▪ 24 µM β-mercaptoethanol

[1] BM Condimed H1* is a supplement for high serum-containing media formulations enhancing the cloning efficiency.
[2] Nutridoma-CS* is a supplement for serum-free medium formulations enhancing the cloning efficiency.

⚠ ***Each medium formulation may contain additional supplements, such as non-essential amino acids and antibiotics, according to individual requirements.***

The concentration of aminopterin in HAT Medium can be gradually reduced with the use of the separate concentrated reagents, for example, HT medium supplement and aminopterin (250x). In this way, aminopterin can be diluted out.

For screening and characterization

- Determination of antibody subtype: IsoStrip Mouse Monoclonal Antibody Isotyping Kit*
- Determination of antibody concentrations using cell culture supernatants: Mouse-IgG ELISA*

For cloning of antibody-producing cells

The media formulations used are the same as for the selection procedure, however, without the presence of HAT- or HT-medium supplement after the selection has been terminated.

For growing of antibody-producing hybridomas

For high serum-containing cell cultures, hybridomas can be grown in any basal medium, such as RPMI 1640 with 5 to 10% FCS (v/v) and additional supplements, such as

- Antibiotics
- L-glutamine
- β-mercaptoethanol
- Sodium pyruvate
- Non-essential amino acids.

For the serum-free culture of antibody-producing hybridomas, choose a Nutridoma preparation according to the hybridoma parent cell line, for example, the myeloma cell line that was used for the fusion, such as Nutridoma-SP* for SP 2/0- derived hybridomas.

1.4. Application

Hybridoma Fusion and Cloning Supplement is a serum replacement, specifically formulated to optimize cell growth of freshly fused hybridomas during selection and cloning procedures in low serum-containing cell culture media.

- HFCS avoids the additional use of serum in cell culture medium for the growth of freshly fused hybridomas derived from SP 2/0, P3X63Ag8.653.
- The specific composition of HFCS furthermore avoids the use of feeder cells. The growth rate of freshly fused hybridoma cells in HFCS-supplemented medium is much higher compared to that in human endothelial culture supernatant (HECS)- or FCS-supplemented medium.
- In cloning procedures of hybridomas, HFCS-supplemented medium is much more efficient compared to FCS-supplemented medium, see section, **Results, Figure 1**.
- HFCS may also be used for the culture of hybridoma cells from species other than mouse (not tested).

2. How to Use this Product

2.1. Before you Begin

General Considerations

Media formulations for the culture of mouse-derived hybridomas

Step	Media		
	High-serum[1]	Low-serum[1]	Serum-free[1]
Fusion	▪ Any basal medium, such as RPMI 1640. ▪ FCS for the resuspension of the cells after fusion.	Any basal medium, such as RPMI 1640.	
Freezing	▪ FCS containing 10% DMSO (v/v).		
Selection[2]	▪ Any basal medium, such as RPMI 1640. ▪ 10% FCS (v/v) ▪ 10% BM Condimed H1 (v/v) ▪ 1x HAT Medium Supplement	▪ Any basal medium, such as RPMI 1640. ▪ 1x HFCS ▪ 1x HAT Medium Supplement	▪ Any basal medium, such as RPMI 1640. ▪ 1x Nutridoma-CS ▪ 1x HAT Medium Supplement
Screening	▪ See **Selection** above.		
Cloning	▪ Any basal medium, such as RPMI 1640. ▪ 10% FCS (v/v) ▪ 10% BM Condimed H1 (v/v)	▪ Any basal medium, such as RPMI 1640. ▪ 1x HFCS	▪ Any basal medium, such as RPMI 1640. ▪ 1x Nutridoma-CS
Hybridoma culture	▪ Any basal medium, such as RPMI 1640. ▪ 10% FCS[3] (v/v)	▪ RPMI 1640/DMEM (1:1) ▪ 0.5 to 1% FCS[3] (v/v) ▪ 1x Nutridoma-CS	▪ RPMI 1640/DMEM (1:1) ▪ 1% Nutridoma-SP[4]

[1] Each medium formulation may contain further supplements, such as antibiotics, L-glutamine, β-mercaptoethanol, sodium pyruvate, non-essential amino acids.
[2] The concentration of aminopterin in HAT-containing medium can be gradually reduced by the use of the separate concentrated reagents, for example, HT medium supplement and aminopterin, (250x). In this way, aminopterin can be diluted out.
[3] For hybridomas to be transferred from serum-containing medium into serum-free medium Nutridoma-SP, weaning is required, see footnote 4.
[4] Nutridoma-SP is recommended for SP 2/0-derived hybridomas.

Working Solution

HFCS, 50x conc. is diluted 1:50 (v/v) with basal medium, such as RPMI 1640.
The final medium should also contain L-glutamine and β-mercaptoethanol.

2.2. Protocols

The following protocols describe the most important steps during the production of hybridomas and monoclonal antibodies from mouse after immunization: fusion, selection, screening, cloning, and hybridoma culture.

See section, **General Considerations** *for a listing of serum-containing and serum-free media for the culture of mouse-derived hybridomas.*

Fusion of cells

Use only myeloma cells that have been tested for the absence of mycoplasma, for example, using the Mycoplasma PCR ELISA*, or DAPI*. In addition, you should routinely test established hybridoma cell lines for mycoplasma infection. To eliminate mycomplasma infections, use the antibiotic combination BM-Cyclin*.

1. In a conical tube, mix 1 × 10^8 mouse spleen cells (in 15 ml serum-free culture medium) with 2 × 10^8 mouse myeloma cells (in 35 ml serum-free culture medium).

2. Spin the cells down for 10 minutes at 300 × *g*.

3. Remove the supernatant with a Pasteur pipette.

   Remove the supernatant completely to avoid dilution of PEG.

4. Gently disrupt the pellet by tapping the bottom of the tube.
   – Place the tube in a +37°C water bath and keep it there during the fusion.

5. Pre-warm 50% PEG 1500 (w/v) to +37°C.
   – Gradually add 1.5 ml pre-warmed 50% PEG 1500 drop-by-drop to the pellet over a period of 1 minute, while continually stirring the cells gently with the pipette tip.

6. Continue to stir the cells for 1 minute.

7. Pre-warm medium, such as RPMI 1640 or PBS to +37°C.
   – While gently swirling the tube, slowly add the pre-warmed medium or PBS at the rate indicated in the table:

Volume [ml]	Time [seconds]
1	>30 to 60
3	>30 to 60
16	>60 to 120

8. Immediately pellet the cells by centrifugation at 300 × *g* for 10 minutes in an uncooled centrifuge.

9. Incubate the centrifuge tube for 5 minutes either at +37°C or at +15 to +25°C.

10. Remove supernatant and gently resuspend the cells with a Pasteur pipette in 10 ml pure FCS.

11. To 10% (1 ml) of the cell suspension, add 4 to 8 ml selection medium, see section, **Selection**.

    This will prepare enough cell suspension for plating in 4 to 8, 24-well cloning plates.

12. Add 1 ml selection medium to each well of a cloning plate.
    – To each well that contains selection medium, add one drop of the cell suspension.

13. Freeze the remaining cells in liquid nitrogen.
    If the cells were resuspended in FCS, add 10% DMSO (dimethylsulfoxide) (v/v) before freezing (approximately 1 ml cell suspension per ampule).

Selection of cells

See section, **Additional Equipment and Reagent Required** *for additional information.*

After fusion, leave cells in selection medium for 7 to 14 days to select for hybridoma cells. Usually the cells must be fed 5 to 7 days after fusion.

Follow the protocol below for feeding:

1. Remove approximately 50% of the culture medium by suction.

2. Add 0.5 to 0.8 ml fresh selection medium.

During this selection period, use a phase contrast microscope to monitor the cells every two days to check for growth, contamination, and the success of the selection procedure. Once the cells have reached an appropriate cell density, in approximately 7 to 14 days, perform an initial screening step to eliminate non-producing hybridomas.

Screening and characterization

Screen hybridomas using the:
- IsoStrip Mouse Monoclonal Antibody Isotyping Kit*, or
- Mouse-IgG ELISA* (coating antibody, AP conjugate, POD conjugate).

Detailed information about the screening procedure is given in the Instructions for Use of each of the products or can be taken from the relevant literature.

Cloning of antibody-producing cells

Once the selection procedure is successful and you have identified positive tissue culture supernatants by screening, the next step is to clone the antibody-producing cells. Single-cell cloning ensures that the antibody-producing cells are truly monoclonal and that the secretion of the antibody can be stably maintained.
There are several methods for single-cell cloning:
- Limiting dilution
- Growth in soft agar
- Flow cytometry

Single-cell cloning by limiting dilution

A protocol for single-cell cloning by limiting dilution is given below. Even though every attempt is made to ensure that the cells are in single-cell suspension prior to plating, there is no way to guarantee that the colonies do not arise from two cells that were stuck together. Therefore, perform limiting dilution cloning at least twice (re-cloning) to generate a clonal population.

Handling instructions

- If many hybridomas have to be cloned at the same time, it may be worthwhile to plate the dilutions by using a 10 ml or larger pipette. One drop from these pipettes will deliver approximately 100 µl.
- Clones will begin to appear in 4 days and should be ready to screen starting approximately days 7 to 10.
- Screens can be done from wells containing multiple clones as well as from wells containing only single clones.

The hybridomas should be healthy and rapidly growing at the time of cloning.

1. Prepare four dilution tubes with medium (with the 3 media described in section, **Selection** without HAT or HT after selection has been terminated) for each cell to be cloned.

   Three tubes should have 2.7 ml and the fourth should have 3.0 ml.

2. Add 10 µl of the hybridoma cells from 24-well cloning plates to the tube containing 3.0 ml of medium.
   – Prepare 1 in 10 dilutions of the hybridomas by removing and transferring 0.3 ml aliquots into the 2.7 ml tubes.

3. Add 100 µl of each dilution into 24 of the wells of a 96-well tissue culture plate (24 wells/dilution; 4 dilutions/ plate, that is, one hybridoma/ plate).

   If the cells from the highest dilution are plated first, then the pipette does not need to be changed during the plating.

Growing of antibody-producing hybridomas

See section, **Additional Equipment and Reagent Required** *for additional information.*

By using Nutridoma-CS supplemented selection and cloning medium directly after fusion (which is performed serum-free in general), the entire procedure for the production of monoclonal antibodies in hybridomas can be done under serum-free conditions.

- During the permanent culture of hybridoma cells, a routine examination regarding qualitative and quantitative antibody production must be performed.
- For qualitative assays, use the same reagents as for the screening/characterization procedure or a functional test. In addition, the subtype of a particular antibody can be easily determined by using the IsoStrip Mouse Monoclonal Antibody Isotyping Kit*.
- For quantitative assays use, for example, the Mouse IgG-ELISA* determination of antibody concentrations in cell culture supernatants.

Use Hybridoma Fusion and Cloning Supplement to culture hybridoma cells from species other than mouse (not tested).*

2.3. Parameters

Biological Activity

Each lot is assayed for high cloning efficiency of a hybridoma cell line, see section, **Results, Figure 1**.

Purity

Endotoxin (LAL): ≤100 EU/ml

Working Concentration

Dilute the 50x-concentrated HFCS 1:50 (v/v) with basal medium. The preferred medium is RPMI 1640.

The final medium should also contain L-glutamine and β-mercaptoethanol.

3. Results

Cloning efficiency of hybridoma cells

A murine B-cell hybridoma was seeded into 96-well cell culture plates at one cell per well. The culture medium used was RPMI 1640 containing 2 mM L-glutamine, 24 µM β-mercaptoethanol, and:

A: 10% FCS, or
B: 1x Hybridoma Fusion and Cloning Supplement (HFCS), or
C: 1x Nutridoma-CS* supplement

12 days later, evaluation was done by recording the positive wells (Fig. 1).

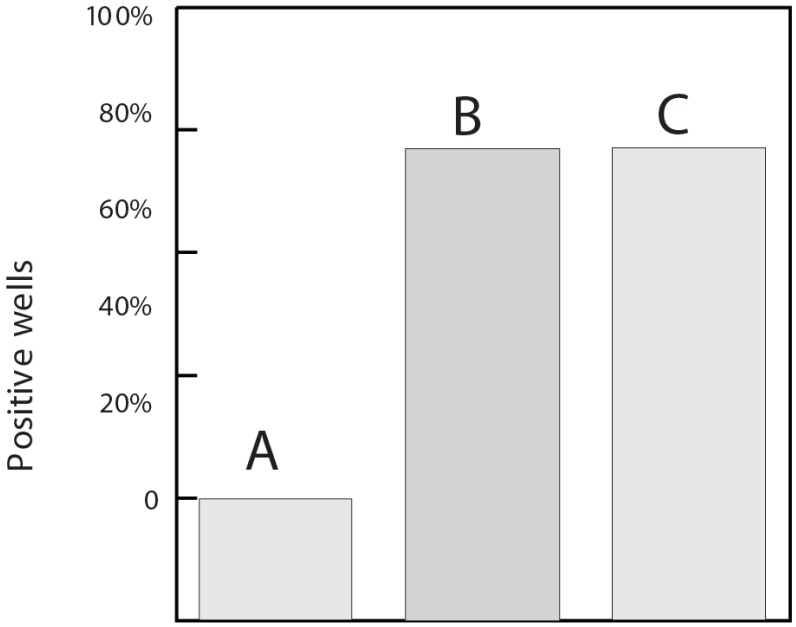

Fig. 1: Improvement of cloning efficiency of hybridoma cells by HFCS and Nutridoma-CS*.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
①②③ etc.	Stages in a process that usually occur in the order listed.
❶❷❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Due to a production change to recombinant transferrin, the section “Safety Information” is removed.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
DAPI	10 mg	10 236 276 001
Nutridoma-SP	100 ml, 100x conc.	11 011 375 001
Nutridoma-CS	10 ml, 50x conc.	11 363 743 001
IsoStrip Mouse Monoclonal Antibody Isotyping Kit	1 kit, 10 tests	11 493 027 001
Polyethylene Glycol 1500	10 x 4 ml	10 783 641 001
BM-Cyclin	37.5 mg, for 2 x 2.5 l medium	10 799 050 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
Product Disclaimers.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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