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Not for use in diagnostic procedures.

Roche

Hybridoma Fusion and Cloning Supplement (HFCS) (50×)

Media supplement
Solution (50× concentrated)

Cat. No. 11 363 735 001

10 ml

Store the solution at −15 to −25°C

Product description

Overview

Hybridoma fusion and cloning supplement (HFCS) is a low serum containing media supplement for the replacement of fetal calf serum (FCS) in cultures of hybridoma cells.
HFCS is specifically formulated to optimize cell growth of freshly fused hybridomas during selection and cloning procedures in low serum-containing cell culture media.
The particular composition of HFCS furthermore avoids the necessity of using feeder cells.

Composition

Medium supplement composed of albumin, insulin, transferrin, cytokines, a cholesterol source, other defined organic and inorganic compounds and fetal calf serum (final concentration: 0.5%).
HFCS contains human proteins. The raw material from which the human proteins were isolated, has been tested for the presence of Hepatitis B surface Antigen (HBsAg) as well as HIV-1 and HIV-2 antibodies and found to be negative.

Biological activity

Each lot is assayed for high cloning efficiency of a hybridoma cell (see Fig.1).

Formulation

- Solution (50× conc., pH 7.4);
- filtered through 0.2 μm pore size membrane;
- endotoxin (LAL): 10 EU/ml, mycoplasma tested

Working concentration

HFCS concentrate (50×) is diluted 1: 50 (v/v) with basal medium. It is strongly recommended to use RPMI 1640. The final medium should also contain L-glutamine and β-mercaptoethanol.

Application

HFCS is a serum replacement which contains FCS and defined quantities of serum albumin, insulin, transferrin, cytokines, a cholesterol source and other specific organic and inorganic compounds.
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 (Fig.1).

Kit storage/ stability

The unopened solution is stable at −15 to −25°C until the expiration date printed on the label.

Note: It is recommended to prepare appropriate aliquots and to avoid repeated freezing and thawing.

Procedures and required materials

Recommended media formulations

In the following table please find the recommended media formulations for the culture of mouse derived hybridomas.

Step	Media formulations		
	high serum[1]	**low serum[1]**	**serum free[1]**
Fusion	• any basal medium (*e.g.*, RPMI 1640) • FCS (for the resuspension of the cells after fusion)	any basal medium (*e.g.*, RPMI 1640)	
Freezing	• FCS containing • 10% DMSO (v/v)		
Selection[2]	• any basal medium (*e.g.*, RPMI 1640) • 10% FCS (v/v) • 10% BM CONDIMED H1 (v/v) • HAT-medium-supplement, 1×	• any basal medium (*e.g.*, RPMI 1640) • 1× HFCS • HAT-medium-supplement, 1×	• any basal medium (*e.g.*, RPMI 1640) • 1× NUTRIDOMA-CS • HAT-medium-supplement, 1×
Screening	• see selection		
Cloning	• any basal medium (*e.g.*, RPMI 1640) • 10% FCS (v/v) • 10% BM CONDIMED H1 (v/v)	• any basal medium (*e.g.*, RPMI 1640) • 1× HFCS	• any basal medium (*e.g.*, RPMI 1640) • 1× NUTRIDOMA-CS
Hybridoma Culture	• any basal medium (*e.g.*, RPMI 1640) • 10% FCS [3] (v/v)	• RPMI 1640 / DMEM (1:1) • 0.5 – 1% FCS[3] (v/v) • NUTRIDOMA-CS	• RPMI 1640 / DMEM (1:1)[4] • NUTRIDOMA-SP 1%

1 Each medium formulation may contain further supplements, *e.g.*, 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 [HT-medium supplement; aminopterin (250×)]. 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 required) (see remark 4).

4 NUTRIDOMA-SP is recommended for SP 2/0 derived hybridomas.

Fusion of cells

Additional reagents required

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

Procedure

Please refer to the following table.

Step	Action
1	Mix 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) in a conical tube.
2	Spin the cells down (10 min, 300 × *g*).
3	Remove the supernatant with a Pasteur pipette. ***Note***: Complete removal of the supernatant is essential to avoid dilution of PEG.
4	• Gently disrupt the pellet by tapping the bottom of the tube. • Place the tube in a 37°C waterbath and keep it there during the fusion.
5	Add 1.5 ml 50% PEG 1500 (w/v), pre-warmed to 37°C to the pellet drop by drop over a period of 1 min, while continually stirring the cells gently with the pipette tip.
6	Continue to stir the cells for 1 min.
7	Add pre-warmed medium (*e.g.*, RPMI 1640) or PBS (phosphate buffered saline) (37°C) as described below, by gently swirling the tube: 1 ml for 30–60 s 3 ml for 30–60 s 16 ml for 60–120 s
8	Immediately spin the cells down (10 min, 300 × *g*) in an uncooled centrifuge.
9	Incubate for 5 min 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 fetal calf serum.
11	To 10% (1 ml) of the cell suspension add 4 – 8 ml selection medium for 4 – 8 cloning-plates with 24-wells.
12	Add one drop of this cell suspension into each well of a cloning plate already containing 1 ml selection medium.
13	Freeze the remaining cells in liquid nitrogen. If the cells were resuspended in FCS, add 10% DMSO (dimethylsulfoxide) (v/v) before freezing (approx. 1 ml cell suspension per ampoule).

Selection of cells

General

After fusion selection of hybridoma cells in selection medium is done for 7 – 14 days.

During this period monitor the cells under a phase contrast microscope every two days with regard to growth, contamination and success of the selection procedure.

Additional media required

Recommended selection media formulations (avoiding the use of feeder cells):
Note: Each medium formulation may contain additional supplements (*e.g.*, non-essential amino acids, antibiotics) according to individual requirements.
The concentration of aminopterin in HAT medium supplement can be gradually reduced with the use of the separate concentrated reagents.

Media	Composition
High serum-containing selection medium	• *e.g.*, RPMI 1640 • 10% FCS (v/v) • 1× HAT-medium-supplement • 10% BM CONDIMED [1] H1* (v/v) • 2 mM L-glutamine • 24 µM β-mercaptoethanol.
Low serum-containing selection medium	• *e.g.*, RPMI 1640 • 1× HFCS • 1× HAT-Medium-supplement • 2 mM L-glutamine • 24 µM β-mercaptoethanol.
Serum-free selection medium	• *e.g.*, RPMI 1640 • 1× NUTRIDOMA-CS[2] * • 1× 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

Procedure

Usually 5–7 days after fusion the cells have to be fed:

Step	Action
1	Remove approx. 50% of the culture medium by suction.
2	Add 0.5-0.8 ml fresh selection medium.
3	Once the cells have reached an appropriate cell density (after 7–14 days) an initial screening step is recommended to delete non-producing hybridomas.

Screening and characterization

General

For the rapid identification of class subclass and light chain type of mouse monoclonal antibodies the ISOSTRIP Mouse Monoclonal Isotyping Kit* or the Mouse Hybridoma Subtyping Kit can be used.
Detailed information about the screening procedure is given in the package inserts.

Cloning of antibody producing cells

Introduction

After selection has been performed successfully and positive tissue culture supernatants have been identified by the first screening, the next step is to clone the antibody producing cells. Single-cell cloning ensures that cells producing the antibody of interest, are truly monoclonal and that the secretion of this antibody can be stably maintained.
There are several methods for single-cell cloning, *e.g.*, by limiting-dilution, growth in soft agar, flow cytometry. A procedure 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, limiting dilution cloning should be done 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 pipet. One drop from these pipets will deliver approximately 100 µl.
- Clones will begin to appear in 4 days and should be ready to screen starting about days 7–10.
- Screens can be done from wells containing multiple clones as well as from wells containing only single clones.

Procedure

Please refer to the following table.

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

Step	Action
1	Prepare four dilution tubes with medium (with the 3 media described under "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 ml of the hybridoma cells from 24-well cloning plates to the tube containing 3.0 ml of medium. Do 1 in 10 dilutions of the hybridomas by removing and transferring 0.3 ml aliquots into the 2.7 ml tubes.
3	Add 100 ml of each dilution into 24 of the wells of a 96-well tissue culture plate (24 wells/dilution; 4 dilutions/plate, i.e., one hybridoma/ plate). ***Note:*** If the cells from the highest dilution are plated first, then the pipet does not need to be changed during the plating.

Growing of antibody producing hybridomas

Additional media required

For high serum-containing cell cultures hybridomas can be grown in any basal medium (*e.g.*, RPMI 1640) with 5 – 10% FCS (v/v) and further supplements, *e.g.*, 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 (i.e. the myeloma cell line that was used for the fusion):

- NUTRIDOMA-SP* is recommended for SP 2/0 derived hybridomas.

Handling instructions

- 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 has to 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 Mouse-Hybridoma-Subtyping Kit*. For quantitative assays use *e.g.*, the mouse IgG-ELISA* determination of antibody concentrations in cell culture supernatants.
- HFCS may also be used for the culture of hybridoma cells from species other than mouse (not tested).

Results

Cloning efficiency

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 10% FCS (A),
- or 1 × HFCS (B)
- or 1 × NUTRIDOMA-CS (C).

12 days later evaluation was done by recording the positive wells.

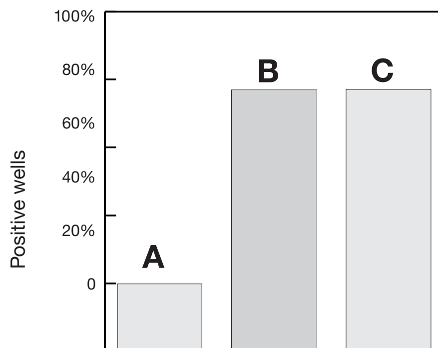

Fig. 1: Improvement of cloning efficiency of hybridoma cells by HFCS and NUTRIDOMA-CS*.

Related products

Kits

Product	Pack Size	Cat. No
IsoStrip Mouse Monoclonal Isotyping Kit	1 kit 10 tests	11 493 027 001
Mouse IgG-ELISA	1 kit 400 tests	11 333 151 001

Single reagents

Product	Pack Size	Cat. No.
BM CONDIMED H1	100 ml	11 088 947 001
Polyethylene glycol 1500	10 × 4 ml	10 783 641 001
NUTRIDOMA-SP	100 ml	11 011 375 001

** available from Roche Diagnostics*

Changes to Previous Version

Editorial changes

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