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Not for use in diagnostic procedures.

BM Condimed H1

Hybridoma cloning supplement
Solution, filtered through 0.2 μm pore size membrane

Cat. No. 11 088 947 001 100 ml

Version 19
Content version: August 2018

Store at −15 to −25°C

1. What this Product Does

Contents

BM Condimed H1 supplement is supplied as a solution, filtered through 0.2 μm pore size membrane in RPMI 1640. The solution also contains 15% FCS (fetal calf serum) (v/v), 1 mM oxalacetate, 1 mM sodium pyruvate, 0.2 μg/ml insulin, 1 ng/ml hIL-6, 10 ng/ml PMA, and phenol red.

Storage and Stability

Stable at −15 to −25°C until the expiration date printed on the label.

⚠ We recommend storing the solution in appropriate aliquots. Avoid repeated freezing and thawing.

Additional Equipment and Reagents Required

Recommended media and reagents for fusion:

- Culture medium: Basal medium, *e.g.,* RPMI 1640 without supplements.
- Polyethylene Glycol, *e.g.,* PEG 1500*.

Recommended selection media (to avoid the use of feeder cells):

- To prepare a **high-serum selection medium,** use *e.g.,* RPMI 1640; 10% FCS (v/v), 1× HAT Medium, 10% BM Condimed H1* (v/v) (a supplement that enhances cloning efficiency in high-serum media), 2 mM L-glutamine, and 24 μM β-mercaptoethanol.
- To prepare a **low-serum selection medium,** use *e.g.,* RPMI 1640, 1× HFCS*, 1× HAT Medium, 2 mM L-glutamine, and 24 μM β-mercaptoethanol.
- To prepare a **serum-free selection medium**, use the components of the low-serum medium (above) but replace HFCS with 1× Nutridoma-CS supplement*, (a supplement that enhances cloning efficiency in serum-free medium).

⚠ You may supplement each medium with additional components (*e.g.,* non-essential amino acids, antibiotics), according to the requirements of your experiment.

To gradually reduce the concentration of aminopterin in HAT medium, combine varying amounts of the separate reagents [HT medium*; aminopterin (250×)].

Application

BM Condimed H1 media supplement is designed for cultivation of freshly fused hybridoma cells in high-serum culture medium.

BM Condimed H1 is added as a supplement (10%, v/v) to normal culture medium (basal medium, *e.g.*, RPMI 1640, DMEM, IMDM) that also contains 10–20% FCS. Such a medium can support the growth of B-cell hybridomas, both after fusion and during cloning. The unique composition of BM Condimed H1 supplement makes feeder cells unnecessary.

BM Condimed H1 supplement should not be used at higher concentrations, as basal medium or as a replacement for serum.

2. How To Use this Product

2.1 Before You Begin

Working Concentration

Add BM Condimed H1 supplement directly to the basal medium at a final concentration of 10%.

2.2 Procedures

The following procedures describe the most important steps (fusion, selection, screening, cloning and hybridoma culture) for producing typical hybridomas and monoclonal antibodies from immunized mice.

Recommended serum-containing and serum-free media for the culture of mouse-derived hybridomas are given in Section 3 of this package insert.

Fusion

⚠ For fusion, use only myeloma cells that have been tested for absence of mycoplasma (*e.g.,* with the Mycoplasma Detection Kit*, 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*.

❶ • In a conical tube, 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).
• Pellet the cells by centrifugation (10 min, 300× *g*).

❷ • Remove the supernatant with a Pasteur pipette.
⚠ You must remove the supernatant completely to avoid dilution of PEG.

❸ • 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.

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

❺ Continue to stir the cells for 1 min.

❻ • Pre-warm medium (*e.g.,* 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 below:

1 ml over 30–60 s
3 ml over 30–60 s
16 ml over 60–120 s

❼ Immediately pellet the cells by centrifuging them at 300 × *g* for 10 min in an uncooled centrifuge.

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

❾ Remove supernatant and gently resuspend the cells with a Pasteur pipette in 10 ml pure fetal calf serum.

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

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

⓫ • 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.

⓬ Freeze the remaining cells in liquid nitrogen. (Use approx. 1 ml cell suspension per ampoule.)

⚠ If you resuspended the cells in FCS, add 10% DMSO (dimethyl sulfoxide) (v/v) before freezing them.

Selection

After fusion, leave cells in selection medium for 7–14 days to select for hybridoma cells. Usually the cells must be fed 5–7 days after fusion. Follow the procedure below for feeding:

❶ Remove approx. 50% of the culture medium from each well by suction.

❷ Add 0.5 - 0.8 ml fresh selection medium to each well.

🔍 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 (after 7–14 days), we recommend performing an initial screening step to eliminate non-producing hybridomas.

Screening and Characterization

- Screen the hybridomas with anti-mouse-Ig Hybridoma Screening Reagent* (coating antibody, POD-conjugate).
- Isotype the monoclonal antibody with the IsoStrip Mouse Monoclonal Isotyping Kit* .

🔍 For detailed information about the screening procedure, see the package inserts of each of the products above or consult the relevant literature.

Cloning

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, *e.g.,* limiting dilution, growth in soft agar, and flow cytometry. The procedure below uses limiting dilution for single-cell cloning.

⚠ Even though you try to ensure that the cells are in single-cell suspension before plating, there is no way to guarantee that the colonies do not arise from two cells that were stuck together. Therefore, you should perform limiting dilution cloning at least twice ("re-cloning") to generate a clonal population.

❶ Make sure hybridomas are healthy and rapidly proliferating at the time of cloning.

❷ For each cell to be cloned, prepare four dilution tubes with medium (a, b or c; without HAT or HT after selection is complete). Three tubes should contain 2.7 ml each and the fourth should contain 3.0 ml.

❸ • Carefully resuspend the hybridomas.
• To the tube containing 3.0 ml of medium, add 10 ml of the hybridoma cell suspension and mix. Use the other three tubes to make serial 1:10 dilutions of the hybridomas.

❹ • Resuspend the hybridomas in each dilution.
• On a 96-well tissue culture plate, add 100 µl of each dilution into each of 24 wells (24 wells/dilution; 4 dilutions/plate, *i.e.,* one hybridoma/plate).

⚠ If you are cloning many hybridomas at the same time, it may be worthwhile to plate the dilutions with a 10 ml (or larger) pipet. One drop from these pipettes will deliver approx. 100 µl.

🔍 If the cells from the highest dilution are plated first, you do not need to change the pipet during the plating.

❺ Clones begin to appear in 4 days and should be ready for screening at about day 7–10.

Growing Antibody-producing Hybridomas

- For culture in high-serum medium, hybridomas can be grown in any basal medium (*e.g.,* RPMI 1640) that is supplemented with 5–10% FCS (v/v) and additional components (*e.g.,* antibiotics, L-glutamine, β-mercaptoethanol, sodium pyruvate, non-essential amino acids).
- For serum-free culture of antibody-producing hybridomas, choose the appropriate Nutridoma preparation based on the hybridoma parent (*i.e.,* the myeloma cell line that was used for the fusion). For example, use Nutridoma-SP supplement* for hybridomas derived form SP 2/0.

🔍 If you supplement the selection and cloning media with Nutridoma-CS supplement, starting directly after fusion (which is generally performed serum-free), the entire procedure (production of monoclonal antibodies in hybridomas) can be done under serum-free conditions.

- During permanent culture of hybridoma cells, you should routinely test them for qualitative and quantitative antibody production.
- For qualitative assays either test the antibodies for function or use the same reagents that you used for the screening/characterization procedure (see *Screening and Characterization*).
- For quantitative assays use *e.g.,* the mouse IgG-ELISA* for rapid determination of antibody concentrations in cell culture supernatants.

🔍 You can easily determine the subtype of a particular antibody with the Mouse Hybridoma Subtyping Kit*.

- You may use HFCS* to culture hybridoma cells from species other than mouse (not tested).

3. Additional Information on this Product

Recommended Media for the Culture of Mouse derived Hybridomas

	High-serum media	Low-serum media	Serum-free
Fusion	– any basal medium (*e.g.,* RPMI 1640) – FCS (for resuspension of cells after fusion)	– any basal medium (*e.g.,* RPMI 1640)	
Freezing	FCS containing 10% DMSO (V/V)		
Selection	– 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 supplement – HAT-medium-supplement, 1×
Screening	see *Selection* above		
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 supplement
Hybridoma Culture	– any basal medium (*e.g.,* RPMI 1640) – 10% FCS3 (v/v)	– RPMI/DMEM (1:1) – 10% FCS (v/v) – 1× Nutridoma-CS supplement	– RPMI1640/ DMEM (1:1) – 1% Nutridoma-SP supplement or Nutridoma-NS supplement (v/v)

How this Product Works

BM Condimed H1 supplement is specifically formulated to optimize growth of B-cell hybridomas during selection and cloning procedures in high-serum culture media. This media supplement is prepared from the supernatant of a mouse thymoma cell line which has been stimulated with PMA. It contains a complex mixture of growth factors and cytokines that stimulate growth of hybridomas after fusion and during cloning (1–3).

Using BM Condimed H1 supplement instead of Feeder Cells

Feeder layer cells from various sources (thymocytes, peritoneal macrophages, splenocytes, irradiated fibroblasts) are widely used to improve the growth of hybridoma cells, both after fusion and during limiting dilution cloning. The major disadvantages of feeder cells are: 1) they may deplete media of nutrients required by growing hybridomas, 2) they sometimes overgrow and kill newly formed hybridomas and 3) they represent a possible source of contamination (4, 5).

BM Condimed H1 supplement eliminates the need for feeder cells and produces more clones after fusion than media containing peritoneal macrophages as feeder cells.

Certain extracts and conditioned media from various sources [*e.g.,* macro-phages cell growth supplement (ECGS), human endothelial culture supernatant (HECS), conditioned media from various cell lines] can replace feeder cell during the critical stages of hybridoma production (6–15). Experiments in our laboratories have shown that media supplemented with BM Condimed H1 supplement produce more clones after fusion than media containng HECS.

References

1 Wu SJ and Ma M. *J. Tissue Cult.* (1985);**9**:147-149.
2 Grabstein K *et al. J. Exp. Med.* (1986);**163**: 1405-1414.
3 Butterfield JH and Weiler D. *In Vitro* (1986);**22**:157-163.
4 Fazekas de St Groth S and Scheidegger D. *J. Immunol.Methods* (1980);**35**:1-21.
5 Galfre´ G and Milstein C. *Methods Enzymol.* (1981);**3:**3-46.
6 Pintus C and Ransom JH *J. Immunol. Methods* (1983)**;61**:195-200.
7 Ransom JH. *Methods Enzymol.* (1981);**121**:293-295.
8 Astaldi GCB *et al.* (1980) J. Immunol. 125, 1411-1414.
9 Astaldi GCB. *Methods Enzymol.* (1983);**92**:39-47.
10 Sugasawara RJ. *et al. J. Immunol. Methods* (1985);**79**:263-275.
11 Rathjen DA and Geczy CL. *Hybridoma* (1986);**5**:255-261.
12 Walker KZ *et al. J. Immunol Methods* (1986);**88**:75-81.
13 Westerwoudt RJ. *Methods Enzymol.* (1986);**121**:3-18.
14 Van Mourik P and Zeijlemaker WP. *Methods Enzymol.* (1986);**121**:174-182.
15 Nordan P and Potter M. *Science* (1986);**233**:566-569.
16 Sweetwyne MT *et al. J. of Histochem. and Cytochem.* (2004);**52**:723-733.

Quality Control

Each lot is tested for its ability to promote the proliferation of freshly fused hybridoma cells.

4. Supplementary Information

4.1 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this package insert:

Text Convention	Use
Numbered Instructions labeled ❶, ❷,etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Diagnostics

Symbols

In this package insert the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

4.2 Ordering Information

	Product	Pack Size	Cat. No.
Associated Kits	Mycoplasma Detection Kit	1 kit (25 tests)	11 296 744 001
	Mycoplasma PCR ELISA	1 kit (96 reactions)	11 663 925 001
	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	Polyethylene Glycol 1500 (PEG 1500)	10× 4 ml	10 783 641 001
	DAPI	10 mg	10 236 276 001
	BM Condimed	100 ml	10 663 573 001
	BM-Cyclin	37.5 mg (for 2× 2.5 l medium)	10 799 050 001
	Hybridoma Fusion and Cloning Supplement (HFCS)	10 ml (50×)	11 363 735 001
	Nutridoma-SP	100 ml	11 011 375 001
	Nutridoma-CS	10 ml	11 363 743 001

4.3 Changes to Previous Version

- Editorial changes.

Trademarks

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