APPENDIX

Appendix A: List of the chemicals and materials used in this study

Chemicals/Materials	Source
Acrylamide	Sigma, St. Louis, MO, USA
Ammonium chloride	Sigma, St. Louis, MO, USA
Ammonium persulfate	Fluka, Buchs, Switzerland
10X BM condimed HI	Roche, Mannheim, Germany
Bovine serum albumin	Sigma, St. Louis, MO, USA
Boric acid	Sigma, St. Louis, MO, USA
Bis-acrylamide	Sigma, St. Louis, MO, USA
Carbon tetrachloride	May & Baker Dagenham, Ikeja Lagos, Nigeria
Cellulose acetate membrane	Helena Laboratories, Beaumont, TX, USA
Coomassie brilliant blue R-250	Bio-Rad, Hercules, CA, USA
DEAE Sepharose	Amersham Biosciences, Uppsala, Sweden
Diethyl ether	Merck, Darmstadt, Germany
Dimethyl sulfoxide (DMSO)	Sigma, St. Louis, MO, USA
Di-sodium hydrogen orthophosphate anhydrous	Fisher Scientific, Cough borough, UK
Disodium salt	England

dNTP	Fermentas, MA, USA
Ethanol	Merck, Darmstadt, Germany
Ethylenediamine tetra acetic acid (EDTA)	BDH Laboratory Supplies, TD, England
Fetal calf serum	Gibco, Gran Island, N.Y., USA
Fungizone (Amphotericin B)	Bristol-Myer Squibb, Cincinnati, OH, USA
Gentamycin	Atlantic Labs, Selangor, Malaysia
Gential violet/ Crytal violet	Sigma, St. Louis, MO, USA
Glacial acetic acid	Merck, Darmstadt, Germany
Glycerol	Merck, Darmstadt, Germany
Hydrochloric acid	Merck, Darmstadt, Germany
50X Hypoxanthine Aminopterin Thymidine (HAT)	Gibco, Gran Island, N.Y., USA
100X Hypoxanthine Thymidine (HT)	Gibco, Gran Island, N.Y., USA
Isocove's Modified Dulbecco's Medium (IMDM)	Gibco, Gran Island, N.Y., USA
Isopropanol	Merck, Darmstadt, Germany
2-mercaptoehanol (2-ME)	Sigma, St. Louis, MO, USA
Methanol	Merck, Darmstadt, Germany
Nonidet P-40	Pierce, Rockford, IL, USA
Paraformaldehyde	Fluka, Buchs, Switzerland
Polyoxyethylenes orbitan monolaurate (Tween 20)	Sigma, St. Louis, MO, USA

Potassium chloride	Merck, Darmstadt, Germany
Potassium cyanide	Reidel-DE Haen AG Sellze-Handnover, Seelze, Germany
Potassium dihydrogen phosphate	Merck, Darmstadt, Germany
Potassium hydrogen carbonate	Fluka, Buchs, Switzerland
Rabbit anti-mouse immunoglobulins	Dako, Glostrup, Denmark
Sodium azide	Reidel-DE Haen AG Sellze-Handnover, Seelze, Germany
Sodium bicarbonate	Merck, Darmstadt, Germany
Sodium carbonate anhydrous	Merck, Darmstadt, Germany
Sodium chloride	Merck, Darmstadt, Germany
Sodium dihydrogen phosphate	Merck, Darmstadt, Germany
Sodium dodecyl sulfate (SDS)	Fisher Scientific, Cough borough, UK
Sodium hydrogen carbonate	Merck, Darmstadt, Germany
Sodium hydroxide	EKA Nobel, Göteborg, Sweden
Taq DNA polymerase and 10X reaction buffer	Fermentas, MA, USA
Tetramethylene ethylenediamine (TEMED)	Sigma, St. Louis, MO, USA
3,3',5,5;-Tetramethylbenzidine (TMB)	Zymed, South san Francisco, CA, USA
Trichloroacetic acid	Fluka, Buchs, Switzerland
Tris base	Amersham Biosciences, Uppsala, Sweden
Trypan blue powder	Sigma, St. Louis, MO, USA

Appendix B: List of instruments used in this study

Instruments	Source
Autoclave	Huxey, Taiwan
Autopipette	Bio-rad, USA
Centrifuge	Kendo Laboratory, Germany
CO_2 incubator	Thermo electron corporation, USA
Electrophoresis and Electrotransfer unit	Amersham,,USA
ELISA reader	Tecan, Austria
Flow cytometer-FACSCalibur	Beckton Dickinson, USA
Inverted microscope	Olympus, Japan
Laminar Flow	NUAIRE, USA
Light microscope	Olympus, Japan
Microcentrifuge	Sorvall, Germany
Multichanel autopipette	Socorex, Switzerland
pH meter	Precisa, Switzerland
Refrigerated centrifuge	Sorvall, Germany
Refrigerat(-20°C)	Sanyo, Thailand
Rotator	Technomara, Switzerland
Spectrophotometer UV-1201	Shimadzu Co., Japan
Thermal cycle	MJ research, INC, USA
UV Transilluminator	Hoefer Scientific Instrument, USA
Water bath	Memmert, Germany

Appendix C: Reagents and buffers preparation

1. Reagents for human blood cell and cell lines culture

1.1 Incomplete IMDM medium

IMDM powder	1 pack
$NaHCO_3$	3.024 g
Gentamycin (40 mg/ml)	1 ml

Dissolved in ddH_20 and adjust volume to 1000 ml

Filtrated through 0.2 µm Millipore membrane filter

Added Fungizone (5 mg/ml)	500 µl

Mixed and stored at 4 ºC

1.2 Complete IMDM medium

Incomplete IMDM medium	90 ml
Fetal calf serum	10 ml

Checked sterility before used

1.3 0.6% 2-mercaptoethanol (2-ME)

Incomplete IMDM	5 ml
2-mercaptoethanol	30 µl

Filtrated through 0.2 µm Millipore membrane filter

Aliquot 50 µl/tube, stored at -20 ºC

1.4 1xHAT medium

Incomplete IMDM	78 ml
Heat inactivated FCS	10 ml
10X BM condimed HI	10 ml
0.6% 2-ME	30 µl
50X HAT	2 ml

Stored at 4 ºC

1.5 1xHT medium

Incomplete IMDM	119 ml
Heat inactivated FCS	15 ml
BM condimed HI	15 ml
0.6% 2-ME	30 µl
100X HT	1 ml

Stored at 4 ºC

1.6 Hypotonic solution (0.083% NH_4Cl) for RBC lysing

NH_4Cl	0.829 g
$KHCO_3$	0.1 g
EDTA	0.0037 g
Deionized distilled water	90 ml

Adjusted pH to 7.2 with 1N HCl

Adjusted volume to 100 ml

Filtrated 0.4 µm Millipore membrane filter

Stored at 4ºC

1.7 Turk's solution

Glacial acetic acid	3 ml
1% gential violet	1 ml

Adjust volume to 100 ml with dH_2O

Filtrated by Whatman filter paper no. 1 and stored at room temperature.

1.8 Trypan blue (0.2%)

Trypan blue powder	0.2 g
PBS pH 7.2	100 ml

Filtrated by Whatman filter paper No. 1 and stored at room temperature.

1.9 Freezing medium (10%DMSO in 25%FCS-IMDM)

Incomplete IMDM	65 ml
FCS	25 ml
DMSO (Hybrimax)	10 ml

Mix well, stored at 4ºC

2. Reagent for PCR

2.1 4M Sodium chloride

NaCl	4.67 g
Sterile distilled water	20 ml.

Mix well, stored at room temperature

2.2 10% Sodium dodecyl sulfate (SDS)

SDS	5 g
Sterile distilled water	50 ml.

Mix well, stored at room temperature

2.3 TE buffer

1M Tris	5 ml.
0.5 M EDTA	10 ml.
Sterile distilled water	485 ml.

Mix well, stored at room temperature

3. Reagent for gel electrophoresis

3.1 1.5% agarose gel

Agarose gel	1.5 g
TBE buffer	100 ml

Heated until dissolved

3.2 10X TBE buffer

Tris (anhydroxymethyl) aminomethane	108 g
Boric acid	55 g
EDTA	9.5 g

Sterile distilled water	1000 ml.

Mix well, stored at room temperature

3.3 Ethidium bromide

Ethidium bromide	50 µl
1X TBE buffer	500 ml.

Mix well, stored at room temperature

4. Reagents for immunoprecipitation

4.1 Tris lysis buffer pH 8.2 (100mM NaCl, 50mM Tris-base, 2 mM EDTA, 0.02% NaN_3)

Tris base	3.03 g
NaCl	2.922 g
EDTA (M.W. 292.25)	0.292 g
NaN_3	0.1 g
Distilled water	200 ml

Adjusted pH to 8.2 by 0.1M NaOH

Adjusted final volume to 500 ml, stored at room temperature

4.2 Lysis buffer

Phenylmethylsulfonyl fluoride (PMSF)	100 µl
(100 mM in acetone)	
Iodoacetamide (0.5M in distilled water)	100 µl

Aprotinin (1 mg/ml in PBS)	100 µl
10% NP40 (in Tris lysis buffer)	1 ml
Tris-lysis buffer pH 8.2	8.7 ml

Mixed well, aliquot to vial and stored at -20 ºC

4.3 1 mM Glycine in PBS

Glycine	0.0375 g
PBS pH 7.2	500 ml

Stored at 4ºC

5. Reagent for SDS-PAGE

5.1 4X Separating gel buffer (1.5M Tris HCl pH 8.8)

Tris base	18.15 g
Deionized distilled water	80 ml

djusted pH to 8.8 by concentrate HCl

Adjusted final volume to 100 ml

Stored at 4ºC

5.2 4X Stacking gel buffer (0.5M Tris HCl pH 6.8)

Tris base	6.0 g
Deionized distilled water	80 ml

Adjusted pH to 6.8 by concentrate HCl

Adjusted final volume to 100 ml

Stored at 4ºC

5.3 10X non-reducing buffer (NRB)

ddH_2O	1.25 ml
1 M Tris-HCl pH 6.8	0.625 ml
Glycerol	1 ml
10% SDS	2 ml
1% Bromphenol blue	125 µl

Aliquot 300 µl/tube, kept at -20 ºC

5.4 5X reducing buffer (RB)

10X NRB	250 µl
2-ME	25 µl
ddH_2O	225 µl

Aliquot 100 µl/tube, kept at -20 ºC

5.5 Running buffer

Tris base	3.028 g
Glycine	14.413 g
Sodium dodesyl sulfate	1.0 g
Distilled water	1000 ml

Mixed well, prepare before use

5.6 30% Monomer (30.8% acrylamide, 2.7% bis-acrylamide)

Acrylamide	60 g
Bis-acrylamide	1.6 g
ddH_2O	200 ml

Mix thoroughly and filtrated through 0.2 µm Millipore membrane filter, kept in dark at 4ºC

5.7 Slab gel

	separating gel			4% stacking gel
	12.5%	10%	7.5%	
Distilled water	3.2 ml	4 ml	4.85 ml	1.5 ml
30% Monomer	4.2 ml	3.3 ml	2.5 ml	332.5 µl
4X Separating gel buffer	2.5 ml	2.5 ml	2.5 ml	-
4X Stacking gel buffer	-	-	-	625 µl
10% SDS (in distilled water)	100 µl	100 µl	100 µl	25 µl
10% APS (in distilled water)	50 µl	50 µl	50 µl	12.5 µl
TEMED	10 µl	10 µl	10 µl	5 µl

5.8 10% APS

Ammonium persulfate	0.1 g
Distilled water	1 ml

Mix well, aliquot and stored at -20ºC

5.9 0.025% Coomassie brilliant blue R250

Coomassie brilliant blue R250	0.125 g
Methanol	200 ml
Acetic acid	35 ml

Adjusted volume to 500 ml by dH_2O

Stored at room temperature

5.10 Destaining gel solution I (40% methanol, 7% acetic acid)

Methanol	400 ml
Acetic acid	70 ml

Adjusted volume to 1000 ml with dH_2O and stored at room temperature

5.11 Destaining gel solution II (5% methanol, 7% acetic acid)

Methanol	50 ml
Acetic acid	70 ml

Adjusted volume to 1000 ml with dH_2O and stored at room temperature

6. Reagents for indirect immunofluorescence staining

6.1 Phosphate buffer saline (PBS)

NaCl	8 g
KCl	0.2 g
Na_2HPO_4	1.15 g
KH_2HPO_4	0.2 g

Distilled water	900 ml

Adjusted pH to 7.2 by 5N NaOH

Adjusted volume to 1000 ml, stored at room temperature

6.2 1%BSA-0.02%NaN_3 in PBS

Bovine serum albumin fraction V	10 g
PBS pH 7.2	1000 ml
10% NaN_3 in PBS	200 µl

Mixed well until BSA completely dissolved, stored at 4ºC

6.3 1%Paraformaldehyde in PBS

Paraformaldehyde	5 g
PBS pH 7.2	500 ml

Heat at 56ºC until dissolved

Filtrated with 0.2 µm millipore filter, stored at 4ºC

7. Reagents for ELISA

7.1 Coating buffer (0.1M carbonate-bicarbonate buffer pH 9.6)

Na_2CO_3	1.06 g
$NaHCO_3$	1.26 g
Distilled water	200 ml

Mixed and adjusted pH to 9.6 with concentrated HCl

Adjusted final volume to 250 ml with distilled water, stored at 4ºC

7.2 0.05% Tween-PBS

PBS pH 7.2	500 ml
Tween 20	250 µl

Mixed and stored at room temperature

7.3 Blocking buffer (2% BSA-PBS)

Bovine serum albumin	2 g
PBS pH 7.2	100 ml

Freshly prepared before used

7.4 Stop reaction solution (1N HCl)

Concentrate HCl	8.3 ml
Distilled water	91.7 ml

Slowly dropwise HCl to distilled water, stored at room temperature

8. Reagents for cellulose acetate electrophoresis

8.1 10X Tri-Borate-EDTA (TBE) buffer pH 8.6 (0.85 M Tris 0.0015 M EDTA 0.055 M Boric acid)

Tris base	121 g
EDTA (disodium salt)	11 g
Boric acid	15 g
ddH_2O	800 ml

Adjusted the pH to 8.6 with saturated Boric acid

Adjusted the volume to 1000 ml with ddH_2O and stored at room temperature

8.2 Working TBE buffer pH 8.6

10X TBE buffer pH 8.6	100 ml
ddH_2O	900 ml

Mix throughly and kept at room temperature

9. Reagents for purification of hemoglobins

9.1 10X Tris-HCl buffer pH 9.0

Tris base	60.57 g
dH_2O	800 ml

Adjusted the pH to 9.0 with 4 N HCl

Adjusted the volume to 1000 ml with dH_2O and stored at room temperature.

9.2 Working Tris-HCl-KCN (THK) buffer pH 9.0

10X Tris-HCl buffer pH 9.0	100 ml
ddH_2O	900 ml
KCN	0.1 g

Mix throughly and filtrated by 0.2 μm Millipore membrane filter, kept at room temperature

9.3 Working THK buffer pH 6.5

10X Tris-HCl buffer pH 9.0	100 ml

ddH_2O	800 ml
KCN	0.1 g

Adjusted the pH to 6.5 with 4 N HCl

Adjusted the volume to 1000 ml with ddH_2O

Mix throughly and filtrated through 0.2 μm Millipore membrane filter, kept at room temperature

Appendix D: Product description of conditioned media

1.1 BM Condimed H1 (Roche)

For life science research only. Not for use in diagnostic procedures. FOR *IN VITRO* USE ONLY.

Roche

BM Condimed H1

Hybridoma cloning supplement
Solution, sterile filtered

Cat. No. 11 088 947 001 100 ml

Version January 2005
Store at −15 to −25°C

1. What this Product Does

Contents

BM Condimed H1 is supplied as a sterile filtered solution 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*, (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 makes feeder cells unnecessary.

BM Condimed H1 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 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* for hybridomas derived form SP 2/0.

Ⓢ If you supplement the selection and cloning media with Nutridoma-CS, 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 – 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
Hybridoma Culture	– any basal medium (*e.g.*, RPMI 1640) – 10% FCS3 (v/v)	– RPMI/DMEM (1:1) – 10% FCS (v/v) – 1× Nutridoma-CS	– RPMI1640/ DMEM (1:1) – 1% Nutridoma-SP or Nutridoma-NS (v/v)

How this Product Works

BM Condimed H1 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 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 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 produce more clones after fusion than media containng HECS.

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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 Applied Science

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

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page www.roche-applied-science.com.

	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

Trademarks

BM Condimed and IsoStrip are Trademarks of a Member of the Roche Group.

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www.roche-applied-science.com/support

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Inspiring Discovery

Roche Diagnostics
Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany

1.2 Hybridoma Cloning Factor (PAA)

PAA
THE CELL CULTURE COMPANY

Protocol for Use

Hybridoma Cloning Factor

Product	Cat. No.	Volume
Hybridoma Cloning Factor	S05-015	50 ml

Hybridoma Cloning Factor is a growth adjunct for cloning and cultivation of Hybridoma Cells.

General
Hybridoma Cloning Factor (HCF) is a partially purified hybridoma growth medium supplement derived from the medium used to cultivate a murine macrophage-like cell line.
HCF increases the cloning efficiency of murine B-cell hybridomas and enhances the growth of hybridomas cultured at low cell densities. It is an ideal replacement for feeder cell layers or other conditioned media currently used for hybridoma cloning or growth during HAT selection, thus eliminating the possibility of contamination from the feeder layer. Many hybridomas which are difficult to grow benefit from the addition of Hybridoma Cloning Factor.
HCF increases the productivity and saves time otherwise spent planning and preparing feeder layers. Preparation of the cloning medium is as simple and easy as diluting the sterile filtered solution of Hybridoma Cloning Factor 10-fold with growth medium (10% final concentration).

Composition
The basal composition of HCF is Iscove's Modified Dulbecco's Medium (IMDM), 2% Foetal Bovine Serum (FBS; heat-inactivated) and kanamycine sulfate.

Shelf Life and Storage
HCF is stable at ≤-15°C for at least two years from date of manufacture. HCF may be frozen and thawed at least five times without appreciable loss of activity; however, it is recommended to aliquot the product into single use volumes so that freeze-thaw cycles will not be required. HCF is stable for 3 days at +2°C to +4°C, room temperature (+22°C), or +37°C.

Hybridoma Growth after Fusion
HCF improves the yield of hybridomas during HAT selection and enhances the number of antibody producing clones.

Instructions for use
1. Perform fusion of splenocytes and myelomas according to established protocol and centrifuge cells to remove the polyethylene glycol.
2. Resuspend the newly fused hybridomas in hybridoma HAT selection medium, i. e. complete IMDM (20% serum, 0.1mM 2-mercaptoethanol, kanamycin and HAT) containing 10% Hybridoma Cloning Factor at a density between $5x10^4$ and $5x10^5$ splenocytes per ml for distribution into 96-well tissue culture treated plates.
   Alternatively, the newly fused hybridomas may be resuspended in the hybridoma growth medium containing Hybridoma Cloning Factor in half the final desired volume and then added to the tissue culture plates. After 18 to 24 hours an equal volume of hybridoma growth medium

PAA
THE CELL CULTURE COMPANY

Protocol for Use

containing 10% Hybridoma Cloning Factor and two times the final concentration of HAT is added to the plates.

3. After ten days of growth (no refeeding of the cultures is necessary), the colonies will be visible to the eye and the supernatant fluids may be assayed for antibody.
4. Antibody positive hybridomas may be expanded in hybridoma growth medium containing 5% Hybridoma Cloning Factor in preparation for freezing and/or cloning.

Hybridoma Cloning

HCF improves the cloning efficiency of hybridomas.

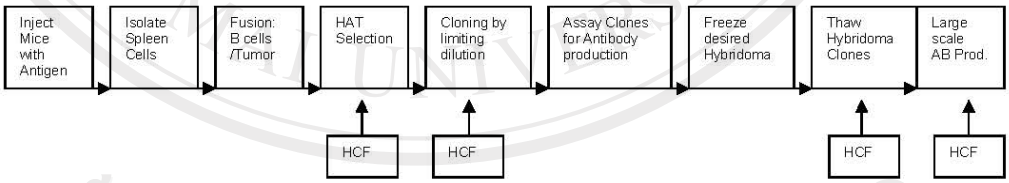
Instructions for use

1. Grow the hybridomas in your cell-specific hybridoma growth medium containing 10% Hybridoma Cloning Factor until cells are in the logarithmic phase (approximately $5x10^5$ cells/ml).
2. Count the cells and dilute them in hybridoma growth medium containing 10% Hybridoma Cloning Factor and at least 4% serum to a density of 5 cells per ml.
3. Distribute 0.2 ml of the cell suspension to each well of a 96-well tissue culture plate.
4. Allow the cells to grow for 10 to 14 days (no refeeding is necessary) and then inspect for macroscopic colonies.
5. Assay the supernatant of wells containing single colonies for antibody.
   Expansions to 24-well plates may be aided by culturing the hybridomas in growth medium containing 5% Hybridoma Cloning Factor.

Growth of Hybridoma Cells after Thawing

HCF enhances the growth of hybridomas or other cells after thawing.
The recovery of viable cells from the freezer is proportional to the initial cell viabilitiy (the number of viable cells/total number of cells). Ideally, the initial pre-freeze percent viability should be greater than 90%. However, this is not always possible. For recovery of frozen hybridoma cells, 5-10% HCF should be added to the culture medium to enhance the growth of hybridomas directly after thawing.

Figure 1: Flow chart Hybridoma Development and Antibody Production



For in vitro laboratory use or further manufacturing only. Not for human use.

1.3 Hybridoma Enhancing Supplement (SIGMA)

3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-576
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

SIGMA®

ProductInformatior

Conditioned Media

CONDITIONED MEDIUM FROM HUMAN GIANT CELL TUMORS (Product No. M 7657)

Bone marrow cell supplement; prepared using a cell line derived from a human histiocytoma, Giant Cell Tumor (GCT) grown in IMDM with 5% FBS.

CONDITIONED MEDIUM FROM EL4-IL2 CELLS, HYBRI-MAX® (Product No. M 8657)

Prepared using a mouse lymphoma cell line, EL4.IL2, grown in IMDM with 2% FBS.

HYBRIDOMA ENHANCING SUPPLEMENT, Thymoma Cell Origin (Product No. H 6020)

Prepared using a proprietary cell line and procedure. It is prepared from a thymoma cell line in RPMI 1640 medium with 15% FBS.

HYBRIDOMA ENHANCING SUPPLEMENT, Lymphoma Cell Origin (Product No. H 2900)

Prepared using a proprietary cell line and procedure. It is prepared from a lymphoma cell line in RPMI 1640 medium with 15% FBS and contains IL-6.

HYBRIDOMA ENHANCING SUPPLEMENT, Macrophage-Like Origin (Product No. H 8142)

Prepared using a proprietary murine macrophagi like cell line grown in IMDM with 2% FBS.

CONDITIONED MEDIUM FROM J774A.1 CELLS, HYBRI-MAX® (Product No. M 8782)

Prepared using a mouse macrophage cell line, J774A.1, grown in IMDM with 2% FBS.

PRODUCT DESCRIPTION

Historically, feeder cell layers or media conditioned by primary cells have been used to increase hybridoma survival, clonal development and the growth of other fastidious cells. Sigma offers six conditioned media, five developed for enhancement of fusion and cloning efficiencies, and one developed to enhance the growth of bone marrow cells.

PRODUCT USE

Conditioned Media are supplied as sterile liquids. A concentration of 10% in complete medium containing 10% FBS or CPSR-3 is recommended. Optimal concentrations should be determined for specific applications.

PRODUCT STORAGE

Product Nos. H 8142, H 2900, M 8782, M 8657 and M 7657 should be stored frozen at −10°C to −30°C. Catalog No. H 6020 may be stored at 2-8°C. Avoid repeated freezing and thawing. If entire contents are not used we recommend preparing smaller aliquots and freezing for future use. Precipitation may occur when stored frozen. This is not detrimental to the product and may be reversed when warmed to 37°C.

1.4 Briclone (QED Bioscience)

QED Bioscience Inc.

ADVANCED RESEARCH TECHNOLOGIES

BriClone

Hybridoma Cloning Medium

Product No.: BRI10000
Size: 100 ml

BACKGROUND

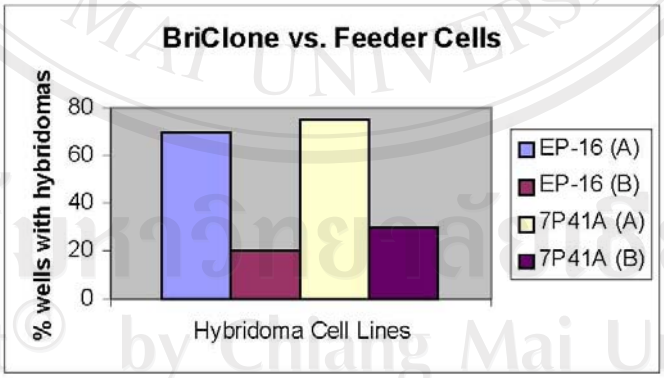
The traditional use of feeder cells to support the outgrowth of newly-created hybridomas has many disadvantages. Batch-to-batch variations may occur in the preparations of feeder cells, feeder cells are a potential source of contamination, and feeder cells may compete for vital nutrients with freshly-fused hybridomas. BriClone hybridoma cloning medium has been developed to overcome and eliminate these common problems.

PRODUCT DESCRIPTION

BriClone is a conditioned medium collected from a proprietary human cell line that produces IL-6. It is supplied as a sterile frozen medium in 100 ml volumes. Each batch of BriClone is tested for its ability to support the outgrowth of hybridomas from freshly fused cells and for its ability to support the growth of hybridomas under conditions of limiting dilution. BriClone is guaranteed *Mycoplasma*-free.

INSTRUCTIONS FOR USE

Add BriClone Hybridoma Cloning Medium to hybridoma culture medim as a 5% (v/v) supplement. BriClone is suitable for use in the post-fusion stages of hybridoma production and when cloning hybridoma cell lines under conditions of limiting dilution. BriClone, when added to culture medium, eliminates the need for feeder cells in these types of cultures.



Hybridoma cell lines EP-16 and 7P41A were cloned by limiting dilution under conditions designed to seed 1 cell/well in 96-well plates. (A) = with 5% BriClone, (B) = with murine macrophages as feeder cells.

STORAGE AND STABILITY

Store at -20°C for up to six months.

This product is intended for *in vitro* research use only. BriClone is a product of Archport Ltd., Dublin, Ireland.

10919 Technology Place, Suite C San Diego, California 92127 Tel 800-929-2114/858-675-2405 Fax 858-592-1509
email info@qedbio.com www.qedbio.com

1.5 Nutridoma CS (Roche)

For life science research only. Not for use in diagnostic procedures.
FOR *IN VITRO* USE ONLY.

Nutridoma-CS (50 ×)

Serum-free media supplement
solution (50 × concentrated),
sterile

Cat. No. 11 363 743 001
10 ml

Version September 2005
Store at −15 to −25°C

Introduction

Nutridoma-CS is a biochemically defined medium supplement for the replacement of fetal calf serum (FCS) in cultures of hybridoma cells.

Nutridoma-CS is specifically formulated to optimize cell growth of freshly fused hybridomas during selection and cloning procedures in serum-free cell culture.

The particular composition of Nutridoma-CS furthermore avoids the necessity of using feeder cells.

Product description

Composition
Biochemically defined serum-free medium supplement composed of albumin, insulin, transferrin, cytokines, a cholesterol source and other defined organic and inorganic compounds. Nutridoma-CS 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) and HIV-1 /2 antibodies and found to be negative.

Biological activity
Each lot is assayed for high cloning efficiency of a hybridoma cell line (fig.).

Formulation
Solution (50× concentrated, pH 7.4); sterile [endotoxin (LAL): <100 EU/ml, mycoplasma tested].
The protein concentration is less than 1 mg/ml for 2% (1×) working concentration.

Working concentration
Nutridoma-CS 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.

Stability
Nutridoma-CS is stable at −15 to −25°C.
It is recommended to prepare appropriate aliquots and to avoid repeated freezing and thawing.

Application

Nutridoma-CS is a serum replacement which contains defined quantities of serum albumin, insulin, transferrin, cytokines, a cholesterol source and other specific organic and inorganic compounds.
Nutridoma-CS completely replaces serum in cell culture medium for the growth of freshly fused hybridomas derived from SP 2/0, P3X63Ag8.653 and NS -1 myeloma cell lines.
The specific composition of Nutridoma-CS furthermore avoids the use of feeder cells.
The growth rate of freshly fused hybridoma cells in Nutridoma-CS supplemented medium is much higher compared to that in human enclothelial culture supernatant (HECS)- or FCS-supplemented medium.
In cloning procedures of hybridomas Nutridoma-CS supplemented medium is much more efficient compared to FCS-supplemented medium (fig.).
Nutridoma-CS is very easy to use. Basal medium supplemented with 1× Nutridoma-CS, 2 mM L-glutamine, and 24 µM β-mercaptoethanol, is used in the same way as serum-supplemented medium formulations. Additional supplements are not necessary (with the exception of HAT-supplements for selection procedures).

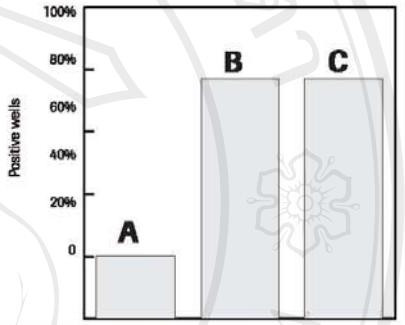


Fig.: Improvement of cloning efficiency of hybridoma cells by Nutridoma-CS and HFCS
A murine B-cell hybridoma was seeded into 96-well cell culture plates at one cell per well. The culture medium used was RPMI 1640 containing 2 mM L-glutamine, 24 µM β-mercaptoethanol, and 10% FCS (A), 1× Nutridoma-CS (B) or 1× hybridoma fusion and cloning supplement (HFCS) (C). 12 days later evaluation was done by recording the positive wells.

A detailed description for the use of Nutridoma-CS in hybridoma culture is given below.

Working instruction

The following working instruction describes as an example the most important steps during the production of hybridomas and monoclonal antibodies from mouse after immunization (fusion, selection, screening, cloning and hybridoma culture). Recommended media formulations for the serum-containing and serum-free culture of mouse-derived hybridomas are given in table 1.

Nutridoma-CS is used in the selection (step 2) and cloning (step 4) procedures of this protocol.

1. Fusion

A. Solutions
- Culture medium: Basal medium, *e.g.*, RPMI 1640 without additional supplements.
- Polyethylene glycol, *e.g.*, PEG 1500* or PEG 4000*.

B. Procedure
- Mix 10^8 mouse spleen cells (in 15 ml serum-free culture medium) with 2×10^8 mouse myeloma cells (in 35 ml serumfree culture medium) in a conical tube.
- Spin the cells down (10 min, 300 × *g*).
- Remove the supernatant with a Pasteur pipette. Complete removal of the supernatant is essential to avoid dilution of PEG.
- 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.
- Add 1.5 ml 50% PEG 1500 or PEG 4000 (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.
- Continue to stir the cells for 1 min at 37°C.

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Roche

- Add pre-warmed medium (*e.g.*, RPMI 1640) or PBS (37°C) as described below, continually but gently swirling the tube:
  1 ml for 30 – 60 s
  3 ml for 30 – 60 s
  16 ml for 60 – 120 s
- Immediately spin the cells down (10 min, 300 × *g*) in an uncooled centrifuge.
- Incubate for 5 min at 37°C or at 15 to 25°C.
- Remove supernatant and gently resuspend the cells with a pasteur pipette.
- If high serum-containing cell culture is desired, resuspend the cells in 10 ml pure fetal calf serum (FCS).
- If low serum-containing or serum-free culture is desired, resuspend the cells in 10 ml Nutridoma-SR.
- To 10% (1 ml) of the cell suspension add 4 – 8 ml selection medium (see section 2) for 4 – 8 24-well cloning-plates.
- Add one drop of this cell suspension into each well of a cloning plate already containing 1 ml selection medium (see section 2).
- Freeze the remaining cells in liquid nitrogen.
- If the cells were resuspended in FCS, add 10% DMSO (dimethylsulfoxide) (v/v), if the cells were resuspended in Nutridoma-SR, add 5% DMSO (v/v) before freezing (approx. 1 ml cell suspension per ampule).

2. Selection

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.

A. Solutions

- Recommended selection media formulations (avoiding the use of feeder cells):

a) To prepare a high serum-containing selection medium use *e.g.*, RPMI 1640, 10% FCS (v/v), 1 × HAT-medium-supplement, 10% BM-Condimed H1* (v/v) (a supplement for high serum-containing media formulations enhancing the cloning efficiency), 2 mM L-glutamine, and 24 µM β-mercaptoethanol.

b) To prepare a low serum -containing selection medium use *e.g.*, RPMI 1640, 1 × HFCS (low serum containing cloning supplement which avoids additional requirement of sera in selection and cloning media and enhancing the cloning efficiency). 1 × HAT-medium-supplement, 2 mM L-glutamine, and 24 µM β-mercaptoethanol.

c) To prepare a serum-free selection medium use the same medium formulation as described under b) but replace HFCS by 1 × Nutridoma-CS.

Note:
Each medium formulation (a, b, c) may contain additional supplements (*e.g.*, non-essential amino acids, antibiotics) according to individual requirements. The concentration of aminopterin in HAT medium can be gradually reduced with the use of the separate concentrated reagents [HT-medium-supplement; aminopterin (250×)]. In this way aminopterin can be diluted out.

B. Procedure

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

- Remove approx. 50% of the culture medium by suction.
- Add 0.5 – 0.8 ml fresh selection medium.

Once the cells have reached an appropriate cell density (after 7 – 14 days) an initial screening step is recommended to delete non-producing hybridomas.

3. Screening

Screening of hybridomas can be done by using the MouseHybridoma-Screening Kit* (ELISA or immunofluorescence assay) or the mouse-hybridoma-screening reagents* (coating antibody, AP-conjugate, POD-conjugate).
Detailed information about the screening procedure is given in the pack inserts of each of the products or can be taken from the relevant literature.

4. Cloning

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.

A. Solutions

- The media formulations used are the same as for the selection procedure (step 2, medium a, b or c), however, without the presence of HAT or HT after the selection has been terminated.

B. Procedure

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

- Prepare four dilution tubes with medium (a, b or c; 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.
- Add 10 µl 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.
- Add 100 µl 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). If the cells from the highest dilution are plated first, then the pipet does not need to be changed during the plating.

Note:

- If many hybridomas are being 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 approx. 100 µl.
- Clones will begin to appear in 4 days and should be ready to screen starting about days 7 – 10.
- Screens can be clonefrom wells containing multiple clones as well as from wells containing only single clones.

5. Growth of antibody producing hybridomas

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.
- Nutridoma-NS* is recommended for NS-11 and P3X63Ag8.653 derived hybridomas.
- Nutridoma-SR is recommended especially for hybridomas to be transferred directly from serum-containing into serumfree culture media to avoid weaning procedures or if a higher protein concentration within the medium is desired.

Note:

- 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 serumfree conditions.
- During the permanent culture of hybridoma cells a routine examination regarding qualitative and quantitative antibodyproduction has to be performed. For qualitative assays use the same reagents as for the screening procedure (see section 3) or a functional test. In addition, the subtype of a particular antibody can be easily determined by using the Mouse-Hybridoma-Subtyping Kit*.
- For quantitatives assays use *e.g.*, the mouse-IgG-ELISA* for the fast determination of antibody concentrations in cell culture supernatants.
- Nutridoma-CS may also be used for the culture of hybridoma cells from species other than mouse (not tested).

Tab. 1: Recommended media formulations for the culture of mouse derived hybridomas

Step	high serum-containing[1]	low serum-containing[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) • Nutricloma-SR (for the resuspension of the cells after fusion)	
Freezing	FCS containing 10% DMSO (v/v)	• Nutridoma-SR containing 5% DMSO (v/v)	
Selection[2]	• any basal medium (*e.g.*, RPMI 1640) • 10% FCS (v/v) • 10% BM Condi-med H1 (v/v) • 1× HAT Medium Supplement	• any basal medium (*e.g.*, RPMI 1640) • 1 × HFCS • 1 × HAT Medium Supplement	• any basal medium (*e.g.*, RPMI 1640) • 1 × Nutridoma-CS • 1 × HAT Medium Supplement
Screening	• see Selection		
Cloning	• any basal medium (*e.g.*, RPMI 1640) • 10% FCS (v/v) • 10% BM Condi-med 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 (v/v) • 1× HFCS	• RPMI 1640/ DMEM (1:1) • 1% Nutridoma-SP, -NS, -SR[4].

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-madiumsupplement; aminopterin (250×)]. In this way aminopterin can be diluted out.

3 For hybriclomas to be transferred from serum-containing medium into serum-free medium either Nutridoma-SR, (no weaning required) or Nutridoma-SP or Nutridoma-NS is recommended (weaning required) (see remark 4).

4 Nutridoma-SP is recommended for SP 2/0 derived hybridomas.
Nutridoma-NS is recommended for NS-1 and P3X63Ag8.653 derived hybridomas, respectively.
Nutridoma-SR is recommended for any hybridoma to be transferred from serum-containing culture to serum-free culture without weaning and/or if a higher protein concentration in the medium is desired.

* *available from Roche Applied Science*

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CURRICULUM VITAE

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2005		Bachelor Degree of Science (Medical Technology), Faculty of Allied Health Sciences, Naresuan University
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Poster Presentation :

สุพรรยา ปาต๊ะ, **นภาพร อภิรัฐเมธีกุล**, วัชระ กสิณฤกษ์. การผลิตโมโนโคลนอลแอนติบอดีต่อโปรตีนบนผิวเม็ดเลือดขาวโดยการฉีดกระตุ้นด้วย immunoprecipitated beads ณ โรงแรมอิมพีเรียลแม่ปิง อำเภอเมือง จังหวัดเชียงใหม่ วันที่ 29 พฤศจิกายน 2549 - 1 ธันวาคม 2549