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Roche

Nutridoma-SP

Serum-free media supplement
Solution, 100× concentrated, filtered through 0.2 μm pore size membrane

Cat. No. 11 011 375 001 100 ml

Version 07
Content version: April 2016

Store at +15 to +25°C
Store protected from light!

1. What this Product Does

Contents

100 ml solution (100× concentrated, pH 7.4); filtered through 0.2 μm pore size membrane.

Formulation and Purity

The solution contains the pH indicator phenol red and appears clear.
[endotoxin (LAL): ⩽10 EU/ml; mycoplasma-tested].

Storage and Stability

Store undiluted Nutridoma supplement (100×) at +15 to +25°C, protected from light.

⚠ Do not refrigerate or freeze the concentrate, as irreversible precipitation of ingredients will occur.

Nutridoma supplement working solutions, *e.g.,* media containing 1% Nutridoma supplement (1%) are stable for 4 weeks when stored at +2 to +8°C in the dark. Do not freeze.

⚠ Do not store in plastic as adsorption of ingredients to the plastic surface may occur.

Application

For the serum-free cultivation of murine myelomas and hybridomas that have an intact cholesterol biosynthesis pathway, such as those derived from the SP2/0, and some of the P3x63Ag8.653 cell lines and lymphoblastoid cell lines, as well as for primary lymphoid cell cultures. It is also used for the serum-free culture of a variety of other cell types, including neural explants.

2. How to Use this Product

2.1 Before you Begin

While the growth of cells in serum-free conditions has many advantages, the following suggested procedures should be considered if optimum product expression and growth is to be achieved.

2.1.1 Weaning of Cells to a Serum-free Environment

Many cell cultures will require weaning from serum-containing medium into a Nutridoma serum-free media. Weaning of cells cultured in serum-containing media is necessary in order to select metabolic pathways required for optimal growth in Nutridoma supplement. The selection (weaning) process may require several passages of the cells through decreasing concentrations of serum.

2.1.2 Optimal basal medium

Various basal media have been formulated to meet the nutritional needs of different cell lines. Nutridoma supplement offers flexibility because it can be used with several basal media. The user can determine the most effective conditions for culturing a particular cell line simply by testing a few preformulated basal media in the presence of 1% Nutridoma supplement. This analysis is most conveniently performed following the serum weaning process.

2.1.3 Low Protein Environment of Nutridoma Supplement

The relatively high protein content of a serum-containing media will bind many small molecules and ions. The reduced protein content of a Nutridoma serum-free medium binds these small molecules and ions to a much lower degree. This can make the cells more susceptible to low concentrations of impurities, additives, cytotoxins, and change of pH. To minimize potential problems associated with these parameters:

- Use only high quality basal medium and water when preparing Nutridoma serum-free medium.
- Use additives (*e.g.,* antibiotics, mitogens, drugs) in lower concentration in serum-free conditions than in the presence of serum. The optimal amount to use will require experimental determination.
- Monitor culture pH, cell viability, and growth phase closely to determine when cells require passage. Typically, cells will have to be subcultured every 2 – 4 days.

2.1.4 Atypical Cell Morphology and Behavior

Cells grown in serum-free media often display atypical morphology and behavior. Compared with most lymphoid-derived cells grown in serum, perfectly healthy cells in Nutridoma supplement may:

- be rounder and have smoother membranes,
- attach less strongly to culture vessel surfaces, and
- be more fragile and susceptible to damage.

These changes in appearance and behavior are independent of each other and in no way reflect a change in cell viability. However, since cells tend to be more “fragile” they require more gentle handling during manipulations.

2.2 Procedures

Use of Nutridoma supplement can be viewed as a three-step process:

- adapt cells to Nutridoma supplement,
- express antibody, and
- freeze cells for future use.

The procedures given below are our recommendations for optimal use of Nutridoma supplement. It should be remembered that not all cell lines will behave or grow the same in Nutridoma supplement (just as in serum). In some cases, optimal use of Nutridoma supplement will require experimentally determined changes to the procedures given. Also, not all cell lines will adapt to serum-free conditions. In these rare instances, Nutridoma supplementcan be used as a serum-extender (1). In these cases, growth of the cells in 1% Nutridoma supplement with the minimum concentration of serum required is the preferred method. However, it should be remembered that antibody production and cell growth are not linked. Therefore, expression of antibody should be monitored as a separate function.

Working Concentration

Nutridoma concentrate (100×) is diluted 1:100 (v/v) with sterile basal medium. Use high glucose DMEM/RPMI 1640 (1:1) (R/D medium) for Nutridoma-SP supplement. The final medium should also contain L-glutamine and sodium bicarbonate. The protein concentration is less than 50 μg/ml for 1% working concentration.

2.2.1 Preparation for Nutridoma Supplement Use

Nutridoma supplement is used as a 1% solution. Add Nutridoma supplement (filtered through 0.2 mμ pore size membrane as supplied) to presterilized basal media using aseptic technique. Do not sterile-filter the 100× Nutridoma concentrate or the 1% working solution. Filtration may remove needed components from Nutridoma supplement.

Media containing 1% Nutridoma supplement can be stored at +2 to +8°C until needed.

Basal media: Although we recommend empirical determination of the most effective basal medium for your cell line, we can make some recommendations based on our experiences with some myeloma and hybridoma cells. Try one of these basal media, in addition to the basal medium that you have been using (with serum) to culture your cells:

Nutridoma supplement	Basal media
Nutridoma-SP supplement	1:1 mixture of high glucose DMEM and RPMI 1640; supplement with 2 mM L-glutamine, (R/D medium)

In addition, some cell lines may require supplementation with 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, and/or 5–10 μM 2-mercaptoethanol, to enhance viability in the above basal media combination.

High glucose DMEM or Ham's F 12 used alone with Nutridoma-SP supplement has generally given poor results. In all cases, avoid using Iscove's DMEM (IMDM) media. The high selenium concentration in this basal medium may be harmful to the cells, because Nutridoma supplement already contains selenium.

Additives: Antibiotics, mitogens, growth factors, drugs, and other metabolites can be added to Nutridoma serum-free media. However, because of the low protein content of Nutridoma supplement, we recommend reducing the concentration of these compounds by 25 – 50% from the level used in serum-containing medium. The final optimal concentration should then be determined by further experimentation.

Buffering: A 1% working solution of Nutridoma serum-free medium has a pH of approximately 7.4. Nutridoma supplement also contains the pH indicator phenol red. Slight variations in pH do not affect the quality of Nutridoma supplement as long as the pH remains below 8.0. If this pH is exceeded (indicated by a red-purple color of the concentrate), insulin and other components may precipitate out of solution. **Do not use Nutridoma supplement which is over pH 8.0**.

In an open system, CO_2 is used to maintain the pH of the culture medium. If you are using an open system with Nutridoma supplement and a basal medium mixture, make sure that the CO_2 level in the culture environment falls between that suggested for the individual basal media. For example, DMEM requires 10% CO_2, whereas RPMI requires 5% CO_2. Use 7.5% CO_2 when using a 1:1 mixture of these basal media.

In a closed system, CO_2 is not used to maintain cell medium pH. Nutridoma supplement can be used in a closed system. However, the pH of the medium and the health of the cells must be very closely monitored.

When working with hybridomas there will be occasions when the cells are out of the incubator for extended periods of time. It is, therefore, important to monitor the pH of the Nutridoma culture.

If cells require additional buffering, Hepes (up to 15 mM) can be used. However, addition of any buffer will quickly change the osmolarity of the medium. Therefore, use the lowest concentration of buffer that allows cell growth yet gives added pH buffering.

2.2.2 General Instructions for Handling Cells Grown in Nutridoma Supplement

During adaptation to serum-free conditions (protocols are below), cells are usually more fragile than normal because of the very low protein concentration in the medium. Accordingly, it is advantageous to not centrifuge cells when sub-culturing. Instead, dilute concentrated cells (cells which have grown to a high density) into the appropriate Nutridoma supplement. Once cells are weaned to Nutridoma supplement, they can be centrifuged at reduced speeds.

Cells grown in Nutridoma supplement are usually in suspension, though these cells sometimes grow in clumps. The type of plastic used plays a large role in whether cells grown in Nutridoma supplement will be adherent or not; some cells adhere to some plastics and not to others. If cells are adherent, resuspend them by gently tapping the tissue culture flask or pipetting medium gently over the cells.

Select rapid or gradual adaptation for your cell line

Rapid adaptation is suitable for most SP 2/0 derived hybridoma cell lines.

Rapid adaptation of myeloma and hybridoma cell lines to growth in Nutridoma supplement

1. Addition of Nutridoma supplement to basal medium

Prepare 100 ml of 1% Nutridoma supplement by adding 1 ml Nutridoma supplement to 100 ml of R/D for Nutridoma-SP supplement.

Do not filter Nutridoma supplement. Nutridoma supplement is supplied as a filtered solution.

2. Choosing a cell culture for subsequent seeding

When cells are seeded, their growth rate is usually slow for the first 2 – 24 h. After this lag phase, cells enter a period of exponential growth (the log phase). When cells finally become crowded, they enter the plateau phase, a period of reduced growth. If cells in the plateau phase are seeded (for hybridomas this usually occurs at cell densities ≧1,000,000 cells/ml), they will probably have a much longer lag period.

The recommendations for choosing flasks for seeding in steps 3–5 (below) are based on our experience of culturing many hybridoma and myeloma cell lines. The densities given for selecting flasks probably represent the log phase of growth for your particular cell line. If, however, you encounter problems using these values, we recommend that you plot the growth of your cells to obtain the appropriate density for seeding.

3. First cell passage in 1% Nutridoma supplement

Select a cell culture growing exponentially in FBS-supplemented medium. Culture should be in mid-log phase of growth.

Subculture cells, in 5 – 7 ml aliquots, in medium supplemented with 1% Nutridoma supplement (0% FBS) in 25 cm_2 flasks at cell concentrations of 100,000 cells/ml and 200,000 cells/ml in duplicate.

Tilt flasks at a 20 to 40 degree angle or stand upright and incubate for two days in a 7.5% CO_2 atmosphere at 37°C.

4. Second passage in 1% Nutridoma supplement

Count the cells and select a flask where the cell density is between 500,000 and 1,000,000 cells/ml. Passage cells, in triplicate, at a concentration of

- 100,000 cells/ml if the cell density is about 1,000,000 cells/ml, or
- 150,000 cells/ml if the cell density is between 700,000 and 1,000,000 cells/ml, or
- 200,000 cells/ml if the cell density is between 500,000 and 700,000 cells/ml.
- Tilt the flasks at a 20 to 40 degree angle or stand upright and incubate for 2 days in a 7.5% CO_2 atmosphere at 37°C.

5. Continue sub-culturing the cells three times a week

For myeloma cells, passage cells at a concentration between 100,000 and 200,000 cells/ml every two days or 100,000 cells/ml for growth over the weekend (three days). As cells adapt to serum-free conditions and the growth rate increases, it may be necessary to reduce the passage density to 50,000 cells/ml for growth over the weekend.

For hybridoma cell lines, passage cells at a concentration between 100,000 and 200,000 cells/ml every two days. To maintain cells over the weekend (three days), passage between 50,000 and 100,000 cells/ml.

Cell lines differ in the speed with which they adapt to Nutridoma supplement. Some cell lines adapt immediately, while others require a few weeks of growth in Nutridoma supplement to reach the same growth rate as that obtained with serum-supplemented medium.

Depending on the cell line and the time between passages, the plating density should vary between 50,000 and 200,000 cells/ml. Use low plating densities for fast-growing cells and for cells left for three-day growth intervals. Use high plating densities for slow-growing cells.

For sensitive cell lines it may be necessary to continue tilting the flask, but well-adapted cell lines can be grown without tilting at this stage.

For cell lines which do not adapt to Nutridoma supplement following this procedure, follow the procedure for "Gradual adaption of myeloma and hybridoma cell lines to growth in Nutridoma supplement below.

Gradual adaptation of myeloma and hybridoma cell lines to growth in Nutridoma Supplement

Gradual adaptation to Nutridoma supplement involves weaning cells gradually from FBS-supplemented to Nutridoma supplement in a stepwise fashion. Cells are weaned from FBS-supplemented medium to 2.5% FBS, 1% Nutridoma supplement, then to 1% FBS, 1% Nutridoma supplement, and finally to 0% FBS, 1% Nutridoma supplement.

1. Addition of Nutridoma supplement to basal medium

Prepare 100 ml of 1% Nutridoma supplement by adding 1 ml Nutridoma supplement to 100 ml of R/D for Nutridoma-SP supplement.

Do not filter Nutridoma supplement. Nutridoma supplement is supplied as a sterile solution.

2. Choosing a cell culture for subsequent seeding

When cells are seeded, their growth rate is usually slow for the first 2 – 24 h. After this lag phase, cells enter a period of exponential growth (the log phase). When cells finally become crowded, they enter the plateau phase, a period of reduced growth (1). Cells for seeding should be chosen from a flask whose cells are in the mid-log phase of growth. If cells in the plateau phase are seeded (for hybridomas this usually occurs at cell densities ≧1,000,000 cells/ml), they will probably have a much longer lag period.

The recommendations for choosing flasks for seeding in steps 3–6 (below) are based on our experience of culturing many hybridoma and myeloma cell lines. The densities given for selecting flasks probably represent the log phase of growth for your particular cell line. If, however, you encounter problems using these values, we recommend that you plot the growth of your cells to obtain the appropriate density for seeding.

3. Wean cells to 2.5% FBS, 1% Nutridoma Supplement

To prepare 2.5% FBS, 1% Nutridoma supplement, add 0.5 ml FBS to a 19.5 ml aliquot of 1% Nutridoma-R/D or -F/D supplement.

Select a cell culture growing exponentially in FBS-supplemented medium. Culture should be in mid-log phase of growth.

Subculture the cells, in duplicate, at a cell density of 100,000 cells/ml and 200,000 cells/ml in 2.5% FBS, 1% Nutridoma supplement. Plant the cells in 5-7 ml aliquots using 25 cm^2 flasks.

Tilt the flasks at a 20 to 40 degree angle or stand upright and incubate for 2 days at 37°C in a 7.5% CO_2 atmosphere.

4. Wean cells to 1.0% FBS, 1% Nutridoma Supplement

To prepare 1.0% FBS, 1% Nutridoma supplement, add 0.2 ml of FBS to a 19.8 ml aliquot of 1% Nutridoma-R/D or /F/D supplement.

After the 48-hour incubation in 2.5% FBS, 1% Nutridoma supplement (step 3 above), count the cells and select a flask where the cell density is between 500,000 and 1,000,000 cells/ml for further passage.

If the culture failed to reach a density of a least 500,000 cells/ml, wait an additional 24 hours and recount.

If the culture exceeded a density of 500,000 cells/ml, subculture the cells, in duplicate, at cell concentrations of 100,000 cells/ml and 200,000 cells/ml with medium supplemented with 1% FBS and 1% Nutridoma supplement.

Incubate the flasks, tilted at 20 to 40 degrees or stand upright, for 2 days at 37°C in a 7.5% CO_2 atmosphere.

5. Wean cells to 0% FBS, 1% Nutridoma Supplement

After 48 – 72 hours of incubating cells in 1% FBS, 1% Nutridoma supplement, count the cells and select one of the flasks for further passage (cell density between 500,000 and 1,000,000 cells/ml).

In duplicate, subculture the cells at concentrations of 100,000 cells/ml and 200,000 cells/ml in 0% FBS, 1% Nutridoma supplement.

Incubate the flasks, tilted at a 20 to 40 degree angle or upright, for three days at 37°C in a 7.5% CO_2 atmosphere. Since cells usually grow slower upon their first passage in 0% FBS, 1% Nutridoma supplement, this incubation step can be scheduled for over the weekend.

Depending on how well cells adapt to their first passage to 0% FBS, 1% Nutridoma supplement, one of three alternatives should be followed:

a) If the culture exceeded a density of 600,000 cells/ml, proceed to step 6.

b) If the cell density is between 400,000 and 600,000 cells/ml, continue to grow in the 0% FBS, 1% Nutridoma supplement for an additional 24 hours. Once the cells have reached a density of 600,000 cell/ml, proceed to step 6. If cells fail to reach a density of 600,000 cells/ml, passage in medium supplemented with 0.5% FBS and 1% Nutridoma supplement until cells are growing well. Afterwards, transfer cells back to 0% FBS, 1% Nutridoma supplement for long-term maintenance (step 6).

c) If the cell density is below 400,000 cells/ml, transfer the cells back into 1% FBS, 1% Nutridoma supplement for one or two passages. Then, transfer the cells to 0.5% FBS, 1% Nutridoma supplement for one or two passages. Afterwards, cells can be transferred back to 0% FBS, 1% Nutridoma supplement for long-term culture.

6. Maintain cells in 0% FBS, 1% Nutridoma supplement

Once cells grown in 0% FBS, 1% Nutridoma supplement have exceeded a density of 600,000 cells/ml usually 3 days, see above), subculture the cells, in duplicate, at cell concentrations of 100,000 cells/ml and 200,000 cells/ml with medium supplemented with 0% FBS and 1% Nutridoma supplement.

Continue to subculture the cells two to three times a week. Select rapidly growing cells for passage. Do not let cells overgrow.

By the following week (three passages), the cells should have fully acclimated to Nutridoma supplement.

Depending on the cell line and the time between passages, the plating density should vary between 50,000 and 200,000 cells/ml. Use low plating densities for fast-growing cells and for cells left for three-day growth intervals. Use high plating densities for slow-growing cells.

For sensitive cell lines it may be necessary to continue tilting the flask, but well-adapted cell lines can be grown without tilting at this stage.

Antibody production

After cells have fully acclimated to Nutridoma supplement, evaluate them for antibody production. Rapidly growing cells won't necessarily be the best antibody secretors. Antibody production may appear to decrease immediately following cell passage to fresh medium and then increase dramatically after the first day of culture. This is due to the very low protein concentration in Nutridoma supplement. Nonspecific binding sites on plastic adsorb antibody. Once these sites are filled, antibody accumulates in the medium.

2.2.3 Cryopreservation of cells in Nutridoma supplement

Cells adapted to Nutridoma supplement can be cryopreserved for future use. Nutridoma media supplements contain a lower concentration of protein than animal serum; therefore, a freezing medium containing Nutridoma supplement may require the addition of 2% serum albumin or 10 to 20% serum. A straightforward freezing protocol which has proven successful in the cryopreservation of several hybridoma cell lines is listed below.

Resuspend log phase cells acclimated to 1% Nutridoma supplement into basal medium containing 1% Nutridoma supplement, 2 mg/ml fatty acid free bovine serum albumin (BSA)* and 8% DMSO for a final concentration of 1×10^6 viable cells/ml. Aliquot 1 ml of cell suspension into biofreeze vials. Freeze slowly, for example, insulate. Insulate vials by wrapping with toweling, cotton, or styrofoam, and freeze at –60°C or below. Store below –90°C for long term storage. To recover frozen cells, rapidly thaw in a 37°C water bath and transfer the cell suspension to 4 ml of medium containing 1% Nutridoma supplement containing 2% BSA. Cells then may require re-adaption (weaning) back to growth in 1% Nutridoma supplement. If re-adaption is required, cells should be passaged through decreasing concentrations of BSA until growth in 1% Nutridoma supplement is achieved.

3. Additional Information on this Product

Background Information

Nutridoma supplement is a chemically defined supplement that can completely replace serum in cell culture media. Nutridoma-SP supplement will support the growth of most SP-20 derived lymphoblastoid, myeloma, and hybridoma cell lines (2-7), as well as primary lymphoid cell cultures (8-9). Nutridoma supplement can occasionally be used successfully with non-lymphoid cell lines. For example, some genetically engineered CHO (chinese hamster ovary) cells grow very well in Nutridoma-SP supplement (10). Nutridoma-SP supplement is specifically formulated to optimize antibody production of hybridomas growing in vitro.

Nutridoma-SP supplement contains defined quantities of highly purified serum albumin, transferrin, insulin, and other specified organic and inorganic molecules.

- Use Nutridoma-SP supplement to culture murine SP2/0 myeloma cell lines and their fusion-derived hybridomas.
- Nuridoma-SP does not have a cholesterol source and is unlikely to support growth of NA-1 and P3X63-Ag8.653 myeloma cell lines and their fusion-derived hybridoma.

Nutridoma supplement as a serum extender

Nutridoma serum-free media can support the growth of most lymphoid cells and some non-lymphoid cells derived from various sources. However, some cell cultures cannot tolerate a total absence of serum. In such cases, Nutridoma supplement can be used as a serum extender (10). To find the lowest concentration of FBS required to support cell growth in a Nutridoma supplement, simply follow the weaning procedure outlined above. Nutridoma supplement can also be used as a basal growth factor supplement to which other cell type-specific growth factors are added.

Composition

Biochemically defined serum-free medium supplement composed of albumin, insulin, transferrin, and other defined organic and inorganic compounds. Nutridoma supplement contains no other growth factors, mitogens, hormones, or sterols.

Nutridoma supplement 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 (HTLV-III) antibodies and found to be negative, according to the current quality control procedures.

Biological Activity

Each lot is assayed for its ability to support the growth of the SP2/0 myeloma cell line.

4. Supplementary Information

4.1 Conventions

Text Conventions

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

Text Convention	Usage
Numbered stages labeled ①, ② etc.	Stages in a process that usually occur in the order listed.
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 Instruction Manual, 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 Changes to Previous Version

- Editorial changes

4.3 References

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Trademarks

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