

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



# Nutridoma-SP

 **Version: 09**

Content Version: September 2023

Serum-free media supplement

**Cat. No. 11 011 375 001**    100 ml  
100x conc.

**Store product at +15 to +25°C.**

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# 1. General Information

## 1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Nutridoma-SP, 100x conc.	<ul style="list-style-type: none"> <li>▪ Biochemically defined solution, containing albumin, insulin, transferrin, and other defined organic and inorganic compounds, pH 7.4.</li> <li>▪ Contains no other growth factors, mitogens, hormones, or sterols.</li> <li>▪ Filtered through 0.2 µm pore size membrane.</li> </ul> <p><b>i</b> <i>The solution contains the pH indicator phenol red and appears clear.</i></p>	1 bottle, 100 ml

## 1.2. Storage and Stability

### Storage Conditions (Product)

When stored at +15 to +25°C, the product is stable through the expiration date printed on the label.

Vial / Bottle	Label	Storage
1	Nutridoma-SP, 100x conc.	Store at +15 to +25°C. <b>⚠ Keep protected from light.</b> <b>⚠ Do not refrigerate or freeze the concentrate as irreversible precipitation of ingredients will occur.</b> <b>⚠ Do not store in plastic as adsorption of ingredients to the plastic surface may occur.</b>

### Storage Conditions (Working Solution)

Store Nutridoma-SP working solutions, such as media containing 1% Nutridoma-SP, 4 weeks at +2 to +8°C.

**⚠ Keep protected from light.**

**⚠ Do not freeze.**

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

## 1.3. Additional Equipment and Reagent required

### Standard laboratory equipment

- Sterile pipettes and flasks
- CO<sub>2</sub> incubator
- Water bath

### Basal media

- DMEM
- RPMI 1640

### For weaning of cells

- FCS (fetal calf serum)
- R/D

### For cryopreservation of cells in Nutridoma supplement

- Bovine Serum Albumin Fraction V, fatty acid free\*
- DMSO

### 1.4. Application

Use Nutridoma-SP for the serum-free cultivation of murine myelomas and hybridomas that have intact cholesterol biosynthesis pathways, such as those derived from:

- SP2/0 and several P3X63Ag8 cell lines and lymphoblastoid cell lines.
- Primary lymphoid cell cultures.

It is also used for:

- Serum-free culture of a variety of other cell types, including neural explants.
- Occasional use with non-lymphoid cell lines, such as some genetically engineered CHO (Chinese hamster ovary) cells which grow very well in Nutridoma-SP supplement.
- Due to its specific formulation, optimization of antibody production of hybridomas growing *in vitro*.

**i** *Nutridoma-SP does not have cholesterol source and is unlikely to support the growth of NA-1 and P3X63-Ag8.653 myeloma cell lines and their fusion-derived hybridoma.*

## 2. How to Use this Product

### 2.1. Before you Begin

#### General Considerations

While the growth of cells in serum-free conditions has many advantages, consider the following points if optimum product expression and growth is to be achieved:

#### Weaning of cells to a serum-free environment

Many cell cultures 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 or weaning process may require several passages of the cells through decreasing concentrations of serum.

#### 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.

#### 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, such as antibiotics, mitogens, and 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 to 4 days.

## 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 have different characteristics:

- Rounder and have smoother membranes.
- Attach less strongly to culture vessel surfaces.
- More fragile and susceptible to damage.

**i** *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.*

## Handling cells grown in Nutridoma Supplement

During adaptation to serum-free conditions (see section, **Protocols**), 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.

**i** *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 SP2/0-derived hybridoma cell lines, see section, **Protocols**.

## Nutridoma-SP supplement as a serum extender

Nutridoma-SP 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-SP can be used as a serum extender. To find the lowest concentration of FCS required to support cell growth in a Nutridoma supplement, simply follow the weaning protocol above. Nutridoma-SP can also be used as a basal growth factor supplement to which other cell type-specific growth factors are added.

## Basal media

Determine empirically the most effective basal medium for your cell line. Suggestions are given in the following table. Alternatively, try the basal medium that you have been using (with serum) to culture your cells.

Nutridoma supplement	Basal media
Nutridoma-SP supplement	<ul style="list-style-type: none"> <li>▪ Use a 1:1 mixture of high glucose DMEM and RPMI 1640; supplemented with 2 mM L-glutamine, (R/D medium).               <ul style="list-style-type: none"> <li><b>i</b> <i>Some cell lines may require supplementation with 1 mM sodium pyruvate, 0.1 mM non-essential amino acids, and/or 5 to 10 <math>\mu</math>M <math>\beta</math>-mercaptoethanol to enhance viability in the basal media combination.</i></li> </ul> </li> <li>▪ High glucose DMEM or Ham's F 12 used alone with Nutridoma-SP supplement has generally given poor results.</li> <li>▪ In all cases, avoid using Iscove's DMEM (IMDM) media. The high selenium concentration in this basal medium may be harmful to the cells, since Nutridoma supplement already contains selenium.</li> </ul>

## 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, reduce the concentration of these compounds by 25 to 50% from the level used in serum-containing medium. The final optimal concentration should then be determined by further experimentation.

## 2. How to Use this Product

### 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 <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 >pH 8.0.**

### Using an open system

Here, CO<sub>2</sub> 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<sub>2</sub> level in the culture environment falls between that suggested for the individual basal media.

- DMEM requires 10% CO<sub>2</sub>, whereas RPMI requires 5% CO<sub>2</sub>.
- Use 7.5% CO<sub>2</sub> when using a 1:1 mixture of these basal media.

### Using a closed system

Here, CO<sub>2</sub> 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.

**i** *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, add HEPES up to 15 mM, 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.*

### Safety Information

Nutridoma-SP 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.

### Working Solution

#### Preparation for Nutridoma supplement use

Nutridoma supplement is used as a 1% solution.

- 1 Add Nutridoma supplement (filtered through 0.2 µm pore size membrane as supplied) to pre-sterilized basal media using aseptic technique.

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- 2 Do not sterile filter the 100x Nutridoma concentrate or the 1% working solution.  
– Filtration may remove needed components from Nutridoma supplement.

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- 3 Store media containing 1% Nutridoma supplement at +2 to +8°C until needed.

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#### Addition of Nutridoma supplement to basal medium

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

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

## 2.2. Protocols

### Overview

Use of Nutridoma supplement can be viewed as a 3-step process:

- Adapt cells to Nutridoma supplement
- Express antibody
- Freeze cells for future use.

The protocols provided are general recommendations for optimal use of Nutridoma supplement. Remember, 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 protocols given. Also, not all cell lines will adapt to serum-free conditions. In these rare instances, Nutridoma supplement can be used as a serum extender. 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.

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

**i** For additional information, see section, **Working Solution**.

#### Choosing a cell culture for subsequent seeding

When cells are seeded, their growth rate is usually slow for the first 2 to 24 hours. After this lag phase, cells enter a period of exponential growth (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  $\geq 1,000,000$  cells/ml), they will probably have a much longer lag period.

Choose flasks for seeding in the following 3 protocols (**First cell passage in 1% Nutridoma**, **Second cell passage in 1% nutridoma**, and **Continue sub-culturing the cells three times a week**). 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, plot the growth of your cells to obtain the appropriate density for seeding.

#### First cell passage in 1% Nutridoma supplement

- 1 Select a cell culture growing exponentially in FCS-supplemented medium.

**i** Culture should be in mid-log phase of growth.

- 2 Subculture cells in 5 to 7 ml aliquots, in medium supplemented with 1% Nutridoma supplement (0% FCS) in 25 cm<sup>2</sup> flasks at cell concentrations of 100,000 cells/ml and 200,000 cells/ml in duplicate.

- 3 Tilt flasks at a 20 to 40 degree angle or stand upright, and incubate for two days in a 7.5% CO<sub>2</sub> atmosphere at +37°C.

#### Second cell passage in 1% Nutridoma supplement

- 1 Count the cells and select a flask where the cell density is between 500,000 and 1,000,000 cells/ml.

- 2 Passage cells in triplicate, as described in the table.

Cell density [cells/ml]	Passage to a concentration of [cells/ml]
1,000,000	100,000
700,000 to 1,000,000	150,000
500,000 and 700,000	200,000

- 3 Tilt flasks at a 20 to 40 degree angle or stand upright, and incubate for two days in a 7.5% CO<sub>2</sub> atmosphere at +37°C.

## 2. How to Use this Product

### Continue sub-culturing the cells three times a week

Cell type	Cell density [cells/ml]	Passage time	Comment
Myeloma cells	100,000 to 200,000	Every two days	100,000 cells/ml for growth over the weekend (three days). <i>i</i> 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.
Hybridoma cell lines	100,000 to 200,000	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.

*i* Depending on the cell line and the time between passages, the plating density should vary between 50,000 and 200,000 cells/ml.

Plating density	Use for...
Low	Fast-growing cells and for cells left for three-day growth intervals.
High	Slow-growing cells.

*i* For sensitive cell lines, it may be necessary to continue tilting the flask, however, well-adapted cell lines can be grown without tilting at this stage. For cell lines which do not adapt to Nutridoma supplement following this protocol, see section, **Gradual adaption of myeloma and hybridoma cell lines to growth in Nutridoma supplement**.

### Gradual adaption of myeloma and hybridoma cell lines to growth in Nutridoma supplement

*i* For additional information, see section, **Working Solution**.

Gradual adaptation to Nutridoma supplement involves weaning cells gradually from FCS-supplemented to Nutridoma supplement in a step-wise fashion. Cells are weaned from FCS-supplemented medium to:

- 2.5% FCS, 1% Nutridoma supplement, then to
- 1% FCS, 1% Nutridoma supplement, then to
- 0% FCS, 1% Nutridoma supplement.

### Choosing a cell culture for subsequent seeding

When cells are seeded, their growth rate is usually slow for the first 2 to 24 hours. After this lag phase, cells enter a period of exponential growth (log phase). When cells finally become crowded, they enter the plateau phase, a period of reduced growth. 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  $\geq 1,000,000$  cells/ml), they will probably have a much longer lag period.

*i* 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, plot the growth of your cells to obtain the appropriate density for seeding.

**Wean cells to 2.5% FCS, 1% Nutridoma supplement**

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

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- 2 Select a cell culture growing exponentially in FCS-supplemented medium.
  - i* Culture should be in mid-log phase of growth.

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- 3 Subculture the cells, in duplicate, at a cell density of 100,000 cells/ml and 200,000 cells/ml in 2.5% FCS, 1% Nutridoma supplement.

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- 4 Plant the cells in 5 to 7 ml aliquots using 25 cm<sup>2</sup> flasks.

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- 5 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<sub>2</sub> atmosphere.

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**Wean cells to 1.0% FCS, 1% Nutridoma supplement**

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

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- 2 After the 48-hour incubation in 2.5% FCS, 1% Nutridoma supplement (section, **Wean cells to 2.5% FCS, 1% Nutridoma supplement**), count the cells and select a flask where the cell density is between 500,000 and 1,000,000 cells/ml for further passage.
  - i* 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% FCS and 1% Nutridoma supplement.

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- 3 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<sub>2</sub> atmosphere.

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**Wean cells to 0% FCS, 1% Nutridoma supplement**

- i* Since cells usually grow slower upon their first passage in 0% FCS, 1% Nutridoma supplement, this incubation step can be scheduled for over the weekend.
- 1 After 48 to 72 hours of incubating cells in 1% FCS, 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).

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  - 2 Subculture the cells in duplicate, at a cell density of 100,000 cells/ml and 200,000 cells/ml in 0% FCS, 1% Nutridoma supplement.

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  - 3 Tilt the flasks at a 20 to 40 degree angle or stand upright, and incubate for 3 days at +37°C in a 7.5% CO<sub>2</sub> atmosphere.

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## 2. How to Use this Product

- 4 Depending on how well cells adapt to their first passage to 0% FCS, 1% Nutridoma supplement, follow one of three alternatives:

Cell density [cells/ml]	Protocol
≥ 600,000	Proceed to section, <b>Maintain cells in 0% FCS, 1% Nutridoma supplement.</b>
400,000 to 600,000	Continue to grow in the 0% FCS, 1% Nutridoma supplement for an additional 24 hours. – Once the cells have reached a density of 600,000 cell/ml, proceed to section, <b>Maintain cells in 0% FCS, 1% Nutridoma supplement.</b> <i>i</i> If the cells fail to reach a density of 600,000 cells/ml, passage in medium supplemented with 0.5% FCS and 1% Nutridoma supplement until cells are growing well. Afterwards, transfer cells back to 0% FCS, 1% Nutridoma supplement for long-term maintenance, as described in section, <b>Maintain cells in 0% FCS, 1% Nutridoma supplement.</b>
< 400,000	Transfer the cells back into 1% FCS, 1% Nutridoma supplement for one or two passages. – Transfer the cells to 0.5% FCS, 1% Nutridoma supplement for one or two passages. <i>i</i> Afterwards, cells can be transferred back to 0% FCS, 1% Nutridoma supplement for long-term culture.

### Maintain cells in 0% FCS, 1% Nutridoma supplement

*i* Usually after 3 days of growth in 0% FCS, 1% Nutridoma supplement, cells have exceeded a density of 600,000 cells/ml.

- 1 Subculture the cells in duplicate, at a cell density of 100,000 cells/ml and 200,000 cells/ml in medium supplemented with 0% FCS, 1% Nutridoma supplement.

- 2 Continue to subculture the cells 2 to 3 times a week and select rapidly growing cells for passage.

*i* Do not let cells overgrow. By the following week (three passages), the cells should have fully acclimated to Nutridoma supplement.

- 3 Depending on the cell line and the time between passages, the plating density should vary between 50,000 and 200,000 cells/ml.

Plating density	Use for...
Low	Fast-growing cells and for cells left for three-day growth intervals.
High	Slow-growing cells.

*i* For sensitive cell lines, it may be necessary to continue tilting the flask, however, 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 will not 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.

## Cryopreservation of cells in Nutridoma supplement

Cells adapted to Nutridoma supplement can be cryopreserved for future use.

**i** *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 shown below.

**1** 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  $1 \times 10^6$  viable cells/ml.

**2** Aliquot 1 ml of cell suspension into biofreeze vials.

**3** Freeze slowly; insulate vials by wrapping with towels, cotton, or styrofoam, and freeze at  $-60^{\circ}\text{C}$  or below.

**i** *Store long-term at  $-90^{\circ}\text{C}$  or below.*

**4** To recover frozen cells, rapidly thaw in a  $+37^{\circ}\text{C}$  water bath and transfer the cell suspension to 4 ml of medium containing 1% Nutridoma supplement containing 2% BSA.  
– Cells may then require re-adaption (weaning) back to growth in 1% Nutridoma supplement.

**i** *If re-adaption is required, cells should be passaged through decreasing concentrations of BSA until growth in 1% Nutridoma supplement is achieved.*

## 2.3. Parameters

### Biological Activity

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

### Purity

Endotoxin (LAL):  $\leq 100$  EU/ml, mycoplasma tested.

### Working Concentration

Nutridoma-SP, 100x conc. 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  $\mu\text{g/ml}$  for 1% working concentration.

## 3. Supplementary Information

### 3.1. Conventions

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

Text convention and symbols	
<b>i</b>	<i>Information Note: Additional information about the current topic or procedure.</i>
<b>⚠</b>	<b><i>Important Note: Information critical to the success of the current procedure or use of the product.</i></b>
① ② ③ etc.	Stages in a process that usually occur in the order listed.
1 2 3 etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

### 3. Supplementary Information

## 3.2. Changes to previous version

Change of the permitted endotoxin limit in the Purity Subchapter.

## 3.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Bovine Serum Albumin Fraction V, fatty acid free	50 g, <i>Not available in US</i>	10 775 835 001

## 3.4. Trademarks

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

## 3.5. License Disclaimer

For patent license limitations for individual products please refer to:  
**List of biochemical reagent products** and select the corresponding product catalog.

## 3.6. Regulatory Disclaimer

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

## 3.7. Safety Data Sheet

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

## 3.8. Contact and Support

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

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

