IS GRO™/ IS PRO™

Catalog No. 91103 **IS PRO™ Media**
Catalog No. 91105 **IS GRO™ Media**

PRODUCT DESCRIPTION

IS GRO™ and IS PRO™ are low protein, serum-free media that comprise a dual formulation media system for the growth of hybridoma cells and the production of monoclonal antibodies. IS GRO is intended for the growth phase of hybridoma cultures, IS PRO is intended for the stationary, production phase of hybridoma cultures.

The two formulations can be used individually, or in sequential combination for batch, fed-batch or perfusion cultures. Optimal cell growth and monoclonal antibody secretion rates are obtained when IS GRO and IS PRO are used in sequential combination.

IS GRO and IS PRO are specifically formulated for high density, large-scale production hybridoma cultures. Both formulations have demonstrated excellent cell growth and monoclonal antibody production in a variety of bioreactor types and culture conditions.

IS GRO/IS PRO is supplied as a refrigerated liquid without L-Glutamine in 1 liter bottles. Larger packaging configurations can be arranged as a custom request. The liquid must be supplemented with 12 mM L-Glutamine (60.0 mL of 200 mM L-Glutamine/Liter medium).

PACKAGING OPTIONS

Catalog No.	Description	Size
Catalog No. 91105	IS GRO Liquid without L-glutamine	1 liter
Catalog No. 91103	IS PRO Liquid without L-glutamine	1 liter

PROTEIN CONCENTRATIONS

IS GRO and IS PRO are formulated using only human proteins.

Medium	IS GRO (50 µg/ml)	IS PRO (1 µg/ml)
Proteins	Albumin Transferrin Insulin	Transferrin

All human proteins are tested in accordance with FDA approved methodologies and are found non-reactive for HBsAg, HIV and HCV, and have been screened at the donor level for exposure to CJD. Handle as if potentially infectious by using universal precautions

SUGGESTIONS FOR GROWING CELLS IN IS GRO AND IS PRO

Maintenance of hybridomas in serum-free media generally requires higher cell densities than with serum-containing media. It is recommended that the cell number not be diluted below 5 x 10^4 cells/mL in IS GRO or IS PRO. Optimum inoculation density is 1-2 x 10^5 cells/mL.

When maximal cell density is greater than 1.0 x 10^6 cells/mL and viability greater than 90%, pass cells 1:5 to 1:10 in IS GRO or IS PRO. For long-term cultivation in IS PRO, cells should be passaged 1:5 rather than 1:10 to ensure adequate long-term viability over multiple passages.

Hybridoma cells should generally follow a passage schedule of twice per week in IS GRO or IS PRO. However, it is important to monitor cell number and viability on a daily basis, since cells grown under serum-free conditions are more susceptible to pH changes and toxic substances in the growth medium. If there is a delay in passaging, viability may decrease.

WEANING CELLS INTO IS GRO OR IS PRO

Most hybridoma cell lines do not require extensive weaning prior to growth in IS GRO. However, due to the extremely low protein concentration of IS PRO, some cell lines may require weaning prior to culturing in IS PRO.

Weaning cells from serum containing medium usually involves a step-wise reduction in the percentage of serum used to culture the cells. Cells should be centrifuged from the serum-containing medium and resuspended sequentially in IS GRO or IS PRO containing 5% FBS, 2.5%, 1%, 0.5% and finally in serum-free IS GRO or IS PRO. With each change, the cells should be cultured for one or more passages until good growth is observed. If growth is near normal at the initial serum concentration, some of the intermediate serum concentrations may be eliminated.

STATIC CELL CULTURE MEDIA EVALUATIONS

In evaluating IS GRO and IS PRO, it is important to note that these media were designed for large-scale hybridoma culture. Therefore, some of the most important benefits of these media may not be readily apparent with testing in 24 well microtiter plates or T-flasks. Because IS GRO and IS PRO are unique and nutrient-rich formulations, utmost care should be used when handling the media. Always use fresh media. It is very important that evaluations be set up with adequate aeration. The best setup would be in spinner flasks (100-1000 mL) in a 5-8% CO_2 incubator with good air circulation.

For T-flask evaluations, an appropriate volume (5-10 mL for T-25s) of IS GRO or IS PRO should be added to each flask and the flasks should be placed in a 5-8% CO_2 incubator for 1-2 hours before the addition of cells. Do not overfill flasks with media. Overfilling will reduce gas exchange with the media. Also, leave the cap loose when placing the flask in the incubator to allow for gas exchange.

Inoculate 1-2 x 10^5 viable cells/mL in triplicate T-25 flasks. Perform cell count and viability assays on a daily basis. If using closed systems (capping flask tight), flush with fresh air (CO_2) on a daily basis. Perform assays for antibody production on the final harvest day or on a daily basis.

USE OF IS GRO AND IS PRO IN BIOREACTORS

IS GRO and IS PRO have been successfully used in stirred tank and hollow fiber bioreactors in fed-batch and perfusion culture. Utilization of IS GRO and IS PRO in different bioreactor types requires different strategies.

Stirred Tank

A. Fed-Batch Culture

In a fed-batch stirred tank culture, hybridoma cells in IS GRO should be inoculated at a density of 1-2 x 10^5 cells/mL. Once the cells have reached maximal density (1-3 x 10^6 cells/mL), feeding should be initiated. Feeding can be done with either IS GRO or IS PRO. For maximal antibody production, IS PRO is recommended. To feed, remove 25-75% of the volume from the bioreactor and replace with fresh media (50% volume changes are recommended). Changes should be made frequently, as warranted by cell density and viability. Monitor viable cell density on a daily basis. Feed once maximal density is reached or viability begins to drop. Feeding may be desirable on a daily or every other day basis, depending upon the cell line's metabolic requirements.

B. Perfusion Culture

In a stirred tank set up for cell recycle using hollow fiber filters or some other method of cell retention and cell-free harvest, hybridoma cells should be inoculated at a density of 1-2 x 10^6 cells/mL. Once the cell density reaches 1-2 x 10^6 cells/mL, initiate continuous perfusion feeding. The daily feed rate will directly affect viable cell density.

Perfusion Rate	Estimated Maximal Cell Density
0.5 Vol/day	4-6 x 10^6 cells/mL
1.0 Vol/day	8-11 x 10^6 cells/mL
2.0 Vol/day	12-16 x 10^6 cells/mL

Proportional increases in antibody production will result from increased cell densities.

Once a peak perfusion rate and cell density have been reached, the feed media can be switched from IS GRO to IS PRO for the rest of the culture.

HOLLOW FIBER CULTURE

The unique nature of hollow fiber culture requires slight changes in the use of IS GRO and IS PRO. In a hollow fiber bioreactor with a fixed molecular weight cutoff, IS PRO should be used as the intercapillary (lumenal) feed medium and IS GRO should be used as the extracapillary (cell space) feed medium. Thus, cells should be grown for inoculation in IS GRO. When harvesting, either in a batch or continuous mode, use IS GRO to replenish the medium in the extra capillary space. Inoculation density, feeding and harvesting should be as per standard procedures used for serum-containing cultures.

CAUTION: Most commercially available hollow fiber bioreactors and prepackaged flowpath units contain glycerin and are sterilized by ethylene oxide. Both materials can be toxic to cells. Cells are more susceptible to toxic components when grown in low protein serum-free media. Thus, it is imperative that hollow fiber bioreactors and flowpaths be flushed well with media prior to use. Both the lumenal and extracapillary spaces need adequate flushing. We recommend at least a three fold increase in flushing volume over what is recommended by most hollow fiber bioreactor manufacturers.

STORAGE AND STABILITY

IS GRO and IS PRO liquid without L-glutamine should be stored at 2 to 8°C. All package configurations should be protected from long-term exposure to light.

IS GRO and IS PRO liquid are stable for one year. All media should be used before the expiration date printed on the product label. Do not use if liquid media is cloudy or contains precipitates.

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