IS GRO™ and IS PRO™

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Serum-Free Media for Hybridoma cells

- Catalog Number 91105 IS GRO
- Catalog Number 91103 IS PRO

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.

Features

- Specifically formulated for high density, large scale production hybridoma cell cultures.
- Little or no adaptation required.
- Shelf life of one (1) year when stored at 2-8°C and protected from light.
- Available in 1 L packaging.
- Custom configurations are available.

Performance

In spite of similar growth curves, significant differences were seen in the final monoclonal antibody concentration among the four combinations (Figure 1). The drop in cell density on day 3 in combination #4 (IS GRO/IS PRO) is due to the removal of half the cells during the feeding (Figure 2). The two spinner cultures in which the IS GRO/IS PRO dual formulation system was utilized reached antibody concentrations exceeding 180 mg/mL. In contrast, the spinner inoculated and fed with IS GRO reached an antibody concentration of 130 mg/mL and the spinner inoculated and fed with IS PRO reached an antibody concentration of 150 mg/mL. Thus, the use of the IS GRO/IS PRO dual formulation system had a dramatic effect on monoclonal antibody production, while the method of feeding (100% vs. 50%) did not appear to affect final antibody concentration.

In both the DME/F-12 + 2% FBS and the IS GRO/IS PRO cultures, cell growth was excellent prior to initiation of perfusion (Figures 3 & 4). After initiation of perfusion, the cells in the IS GRO/IS PRO culture grew more rapidly and reached a maximal density of 9 x 10^6 cells/mL (Figure 4), whereas the DME/F-12 + 2% FBS culture reached a maximum of only 6 x 10^6 cells/mL (Figure 3).

Significant differences were observed in monoclonal antibody production between the two cultures (Figures 3 & 4). Average monoclonal antibody concentration in the DME/F-12 + 2% FBS culture was 150 mg/mL. During the IS GRO and IS PRO phases of the IS GRO/IS PRO culture, the average antibody concentrations were 240 mg/mL and 360 mg/mL, respectively. Peak concentrations were 230 mg/mL in the serum culture and 400 mg/mL in the IS GRO/PRO culture. Volumetric productivity in the DME/F-12 + 2% FBS culture was 0.14 mg of antibody per liter of medium consumed, while in the IS GRO/PRO culture it was 0.28 mg/L. Total daily production in the DME/F-12 +2% FBS culture averaged 1.5 g/day (Figure 3). In the IS GRO/PRO culture, daily production averaged 2.4 g/day during the IS GRO phase, and 3.6 g/day during the IS PRO phase.

Figure 1. Spinner Cell Growth Curves

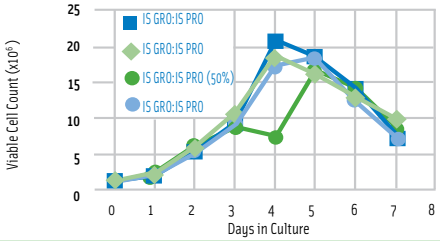
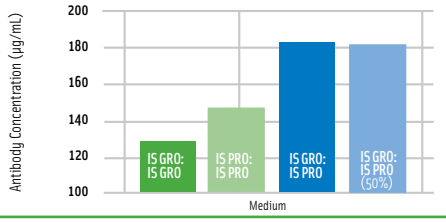


Figure 2. Spinner Culture Final Antibody Concentration



Figures 1 & 2. HFN 7.1 cells were grown in IS GRO. The cells were centrifuged and resuspended in either DME/Ham's F-12 (1:1) plus 10% FBS, IS GRO, or IS PRO. Spinner flasks were inoculated with 900 mL of cells in the appropriate medium at a density of 1-2 x 10^5 cells/mL. All spinner flasks were incubated at 37°C for eight days. All spinners were fed on day three. Daily samples were removed from each spinner for cell counts and monoclonal antibody determination by ELISA. Four growth/feeding combinations were used. They were: 1) IS GRO/IS GRO, 2) IS PRO/IS PRO, 3) IS GRO/IS PRO, 4) IS GRO/IS PRO. In each case the medium was used for days 1-3, then the spinners were fed with the second medium. In combinations 1, 2, and 3, feeding was by centrifugation and resuspension in fresh medium. In combination 4, feeding was by removal of 50% of the volume of the spinner and refilling with fresh medium.

Figure 3. Stirred Tank (DME/F-12, 2% FBS) Viable Cell Count & Production/Day

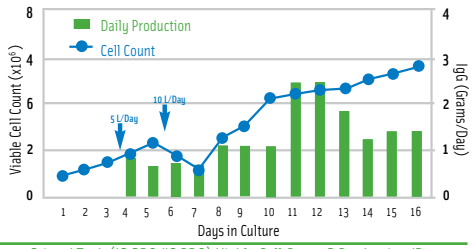
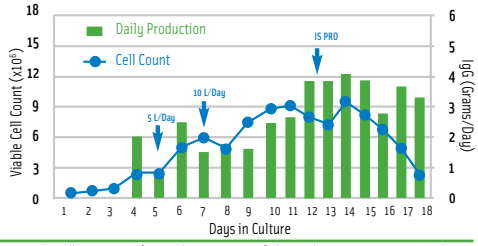


Figure 4. Stirred Tank (IS GRO/IS PRO) Viable Cell Count & Production/Day



Figures 3 & 4. Cells were grown for inoculation in spinner flasks in either IS GRO or DME/Ham's F-12 (1:1) plus 2% FBS. Inoculation density was 1-2 x 10^5 cells/mL. Perfusion at a rate of 5L/day was initiated once the cell density reached 1-2 x 10^6 cells/mL. Perfusion at a rate of 10L/day was initiated once the cell density at 5L/day had peaked. In the IS GRO/IS PRO cultures, the medium was changed from IS GRO to IS PRO after the cell density reached a maximum at a fresh medium perfusion rate of 10L/day. The medium change was accomplished by switching the feed medium container from IS GRO to IS PRO.

Adaptation

HEK 293 cells currently adapted to serum-free medium can be subcultured directly into supplemented IS 293 with minimal adaptation, though a higher initial density should be used. The cells should be in mid-logarithmic growth phase with high (> 90%) viability. Adaptation of HEK 293 cells to serum-free culture conditions may require either direct or sequential adaptation depending upon cell type and culture conditions.

Always refer to product insert for complete instructions for use. For more information on all of our Cell Culture Products, call 1 (800) 437-5706 and ask for your Territory Manager or visit our website at www.irvinesci.com.

North America Headquarters
Irvine Scientific
2511 Daimler Street, Santa Ana, CA 92705
USA
Phone: 1 (949) 261-7800
Toll Free: 1 (800) 437-5706
Fax: 1 (949) 261-6522
www.irvinesci.com

Europe Headquarters
Irvine Scientific
Unit 31, Newton Business Center, Block D
Newtownmountkennedy
County Wicklow
Ireland
Phone: +353 1 281 99 20
Fax: +353 1 281 99 28

Asia Headquarters
Irvine Scientific
3-17-35 Niizo-Minami
Toda-Shi
335-0026 Saitama
Japan

IrvineScientific®