IRVINE SCIENTIFIC

R & D Pilot Study

IS-BEVS Performance Test

IS-BEVS (SERUM-FREE INSECT CELL CULTURE MEDIUM)

Cat. No.:	**99237**	**500 mL**	**Storage Conditioins: +2°C to +8°C, in the dark**
Lot. No.:	**9923750601**		**Expiration: Dec 1995**

IS-BEVS is a serum free medium which supports the growth of *Spodoptera frugiperda* (Sf9, Sf21) and *Trichoplusia ni* (BTI-TN-5B1-4 or High-Five™) cells. This protein free medium supports high production of insect virus and recombinant DNA proteins, and has the capacity for long term cell growth. Cells grown in other serum free medium can be subcultured directly into IS-BEVS with minimal adaptation.

Results

Cell growth data, shown in Figure 1, were obtained from Sf 9 cells grown in IS-BEVS and seeded into 125 mL disposable shake flasks at 3 X 10^5 viable cells/ml in 30 mL of culture medium as indicated. Cultures were incubated at 28°C with shaking at 135 rpm and viable cell density was determined by trypan blue exclusion. Expression studies, shown in figures 2 and 3, were obtained by infecting cells in log phase growth at densities of 1 to 2 X 10^6 cells/ml in 30 mL of medium (in 125 mL disposable shake flasks) with rAcNPV for a) β-Galactosidase (β-gal) at an MOI of 5 or b) secreted alkaline phosphatase (SEAP) at an MOI of 9. Samples of infected culture medium were collected at the indicated times and assayed for β-gal or SEAP activity as indicated in Fig. 2 and Fig. 3. These experiments were repeated a minimum of three times.

Our results indicate that Sf 9 cells grown in IS-BEVS (in shaker cultures) consistantly grow to high cell densities (5 to 6 X 10^6 cells/mL) with greater than 90 % viability. Similar testing with High-Five™ cells indicates growth in a comparable range obtained with several other serum free media formulas (data not shown).

The production of recombinant proteins can be improved in IS-BEVS compared to other media formulas as shown in Figures 2 and 3. Using either Sf 9 or High-Five™ cells, IS-BEVS yielded higher levels of β-gal production than other commonly used formulas (Fig. 2). The production of SEAP in IS-BEVS is consistently better in some cases (Fig 3). The data lead to the conclusions that optimum production is a function of both cell line and recombinant vector/product, and that for many applications IS-BEVS is likely to yield the best results.

Figure 1.

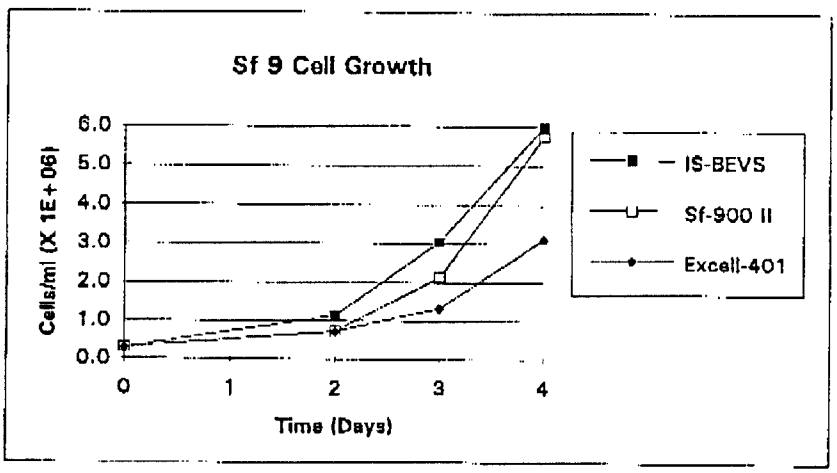


Figure 2.

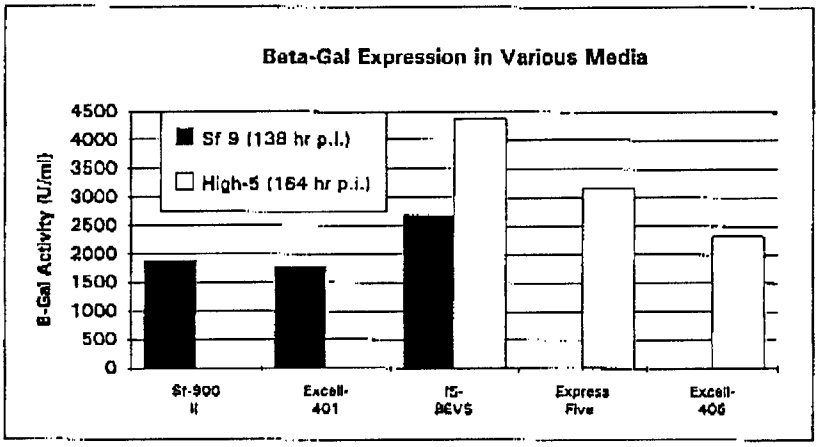
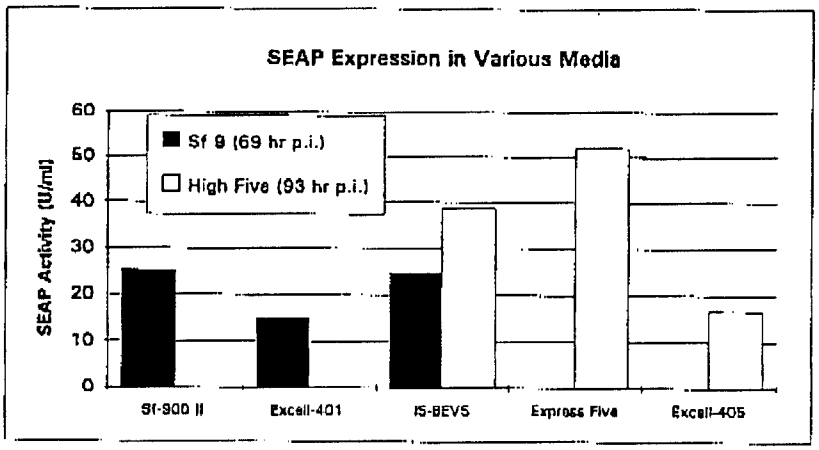


Figure 3.



Cell Culture Procedures

IS-BEVS is complete and ready to use. Please test this medium in the same way that you currently use other serum free insect cell media. We are interested in obtaining additional information regarding the performance of our medium for insect cell growth, virus production and recombinant protein expression for other model systems.

Insect cells currently adapted to serum free medium can be subcultured directly into IS-BEVS with essentially no adaptation. However, we recommend that the cells be grown for a minimum of two (2) passages in IS-BEVS prior to performance testing. Cells should be subcultured two times per week when the density reaches 2 to 4 X 10^6 cells/mL with at least 90% viability, to a subculture density of 3 X 10^5 cells/mL. If the direct adaptation method gives suboptimal performance, sequential (weaning) adaption may be required. Additionally, frozen stock cultures of cells adapted to serum free conditions can be directly recovered in IS-BEVS and should be grown for a minimum of two (2) passages in IS-BEVS prior to performance testing. If cells are currently maintained in medium containing serum, sequential adaptation to serum free medium is required.

If you need any additional information or have questions please contact:

Rebecca Gilbert, Ph.D.
Senior Research Scientist
Research & Development
(800)-437-5706
(rgilbert@ix.netcom.com)

SURVEY and EVALUATION

(R & D Pilot Study 6/95)

Date: _____

Medium formula tested: IS-BEVS Lot # 9923750601

Participating Test Site

Name: Erle S. Robertson (Evaluator) Phone: 617-732-7046

Company: Harvard Medical School Fax: 617-278-6964

Address: BWH ID Division
20 Shattuck St.
Boston, MA 02115

e-mail: esrobert@mbcrr.harvard.edu

What cell line(s) did you use: SF9

Source of cell line(s): Invitrogen

What media do you currently use? Grace

How much do you pay for this medium? _____

What are your growth conditions (please circle appropriate response or fill in): Stationary

1. Stationary or suspension culture
2. Size and type of container: Flask T75 - T225 -
   (Flasks, shakers, spinners, bioreactors, etc.)
3. Volume of culture: 40 - 200 mls
4. Incubator conditions: 27°C NO Co2
5. Other: _____

Analyses/Results (Please attach any charts, graphs, and/or tables which represent your results)

Growth and viability: _____

Morphology (Please note the morphology of cells between media, e.g. single cells, clumpy, etc.): _____

Protein expression (if possible): _____

(OVER)

(Please attach additional sheets as needed)

Additional analyses (virus titer and/or production; recombinant baculovirus vs. wild type AcMNPV):

Comments/Suggestions:

Thank you for your participation.

Please return as soon as possible to:

Irvine Scientific
2511 Daimler St.
Santa Ana, CA 92705-5588
ATTN: **Rebecca Gilbert**