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Cell Culture Application Notes

Application Note No. 1 - General Information/Materials and Methods

Introduction

This series of Application Notes has been developed to assist our customers in the selection and use of Sheffield™ protein hydrolysates for cell culture systems. Since each customer's application requirements are unique with respect to cell lines, expression systems, basal media and culture platforms, this information is only intended to be a guideline. Results may vary in different applications, and individual systems may require further optimization.

For detailed product specifications, see the corresponding Product Information Sheets, available from your Account Representative, or on-line at http://www.SheffieldBioScience.com/.

Stock Solutions and Sterilization

Incorporation of our products into a basal growth medium at laboratory scale is accomplished through the use of concentrated, sterile stock solutions. In our laboratory, these solutions are generally prepared at a concentration of 100-g/l hydrolysate, and added to the basal medium accordingly. For example, if the final desired hydrolysate concentration in the basal medium is 1-g/l, the 100-g/l stock solution is prepared, sterilized, and then added to the basal medium at a rate of 1-ml/l, yielding a final hydrolysate concentration of 1-g/l. The volume of stock solution aliquot should be subtracted from the desired final volume of the basal medium prior to addition of the solution.

To prepare the stock solution, dissolve the hydrolysate in the appropriate basal medium, and then sterilize by passing through a 0.22 μM non-protein binding syringe filter, or a vacuum filter unit for larger volumes. While hydrolysates are freely soluble in most media, in some cases they may be slow to go into solution. This can be overcome by placing the solutions in a 37° C incubator for approximately 30 minutes prior to sterile filtration.

Stocks solutions should not be prepared in water, due to the dilution effect on the final medium formulation. Preparation in buffers is also not advised, as precipitates may form, altering the performance of the product. Heat sterilization of hydrolysate stock solutions is not recommended, due to the risk of precipitation and potential degradation of hydrolysate components.

Stock solutions may be stored at 4°C, with the shelf life determined by the expiration date of the basal medium used as a solvent.

Dosage

The contribution of protein hydrolysates to the overall performance of a biopharmaceutical production system can be influenced by a number of factors including the specific cell line being employed, the raw material used to manufacture the hydrolysate, the hydrolysate dosage, and the composition of the basal growth medium.

Optimal dosages for enhanced growth, viability and protein production in CHO cells have been experimentally determined for a number of Sheffield™ cell culture products. In some cases, these three functional criteria are not correlated. Addition of a hydrolysate may increase maximum cell densities, or improve viability, but not enhance protein production. Conversely, addition of a hydrolysate may suppress growth, but improve viability or significantly enhance protein production.

Dosage rates should be adjusted to reflect the requirements of a particular application. See individual product Application Notes for additional information.

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Osmolarity/Specific Osmotic Increment

Addition of any medium supplement will alter the osmolarity of the basal medium as a function of the particular supplement's composition. The Specific Osmotic Increment (SOI) indicates the expected increase in osmolarity per gram of hydrolysate per liter when added to a cell culture medium.

For example, a product with an SOI of 5.6 mOsm, added to the basal medium at a rate of 5-g/L, will increase the osmolarity of the final medium by 28 mOsm.

The average SOI of each product is indicated on the individual product Application Notes. Further information is available in Application Note No. 4, "Osmolarity and Hydrolysates."

Materials and Methods

Unless otherwise noted, the data presented in the product-specific application notes were collected using the following materials and methods:

Sheffield™ Clone B.1 is a transfected CHO-K1 line engineered to constitutively express secreted embryonic alkaline phosphatase (SEAP) by means of a modified human cytomegalovirus (HCMV) promoter. A sub-clone (KCC-010) of the parent line, which has been adapted to suspension culture in serum-free medium, was used in these experiments.

Cultures were grown in 125 ml shake-flasks containing a final medium volume of 25 ml. The basal media consisted of a commercially available chemically defined medium supplemented with 0.2% Pluronic and 1-mg/ml G-418. Triplicate cultures were seeded at 0.3×10^6 cells/ml, and incubated at 37°C in 5% CO_2 at 130 rpm for 12 days.

Monolayer cultures were grown in a medium consisting of 50% commercially available chemically defined medium, 50% Ham's F12-K, and 5% FBS. Cells were seeded at 0.1×10^6 cells/ml, with a working volume of 3 ml/well in six-well microplates.

Hydrolysate supplementation was achieved via the use of filter-sterilized 100 g/l stock solutions prepared in each respective basal medium. At days 5, 7, 8 and 9, 200 µl of the culture supernatants were removed for assessing cell counts and viability.

Cells were counted using a NucleoCounter fluorescence-based automated cell counter. At Day 12, 200 µl of the culture supernatants were removed for SEAP analysis. Levels of functional SEAP in the supernatants were measured using an absorbance-based activity assay.

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