

XerumFree™ XF212



Subject: VERO cells as substrate for the production of viral vaccines in Chemically Defined (CD) and Animal Component-Free (ACF) cell culture conditions.

BACKGROUND

Vero cells were first separated from a normal adult African green monkey kidney cell line by Yasumura of Chiba University, Japan in 1962.

This cell line presents several advantages over primary and diploid cell substrates:

- Documented Vero cells can be easily accessed from international cell banks
- Vero cells grow fast and require no tedious culture conditions
- They support various virus proliferations
- The oncogenic potential of Vero cells has been extensively characterized. Numerous studies have shown that this cell line is free from oncogenic properties and is not presenting any threat to human health when used as a substrate for biological production (WHO1)
- Vero cells can be grown and expanded in microcarrier and suspension cultures for large scale production in bioreactors
- Vero cells have consistently yielded virus titers that are sufficiently high enough to be considered commercially viable

WHY USE XERUMFREE™ IN VACCINE PRODUCTION PROCESSES ?

During vaccine process development safety is of paramount concern since the majority of viral vaccines are for use in healthy paediatric subjects.

One of the important points of safety concerns relates to the composition of culture medium. All classical vaccine production processes make use of animal derived substances: serum, trypsin,

lactalbumin, etc. Animal sera have for long been considered an essential requirement for vigorous growth of animal cells as a source of nutrients, hormones, growth factors and protease inhibitors. Furthermore, serum also facilitates the attachment and spreading of cells, and provides nonspecific protection against mechanical damage and shear forces. In addition, it is able to bind to toxic compounds. However, besides these growth-promoting properties, serum has some major disadvantages related to its undefined nature with respect to its chemical composition. It can be a source of adventitious agents and their by-products. Serum also presents a variable performance of cell growth and has a substantial cost. For all these reasons, the benefits of in vitro culture of animal cell lines in the absence of serum is now widely acknowledged.

XerumFree™ is a serum-free, ultra-low protein medium manufactured without any components of animal or human origin in order to exclude the potential for contamination by mammalian pathogens and to eliminate all problems associated with the use of animal sera. XerumFree™ has been formulated to achieve consistent cell growth and virus production in laboratory scale applications as well as in large scale production runs, both in adherent and suspension growth systems.

1World Health Organization. Initiative for Vaccine Research. Use of Cell Lines for the Production of Influenza Virus Vaccines: An Appraisal of Technical, Manufacturing, and Regulatory Considerations, WHO, Geneva, 2007.

XerumFree™ Serum Replacement Application Sheet

POINT TO CONSIDER	DESCRIPTION	TNC BIO SUGGESTION	ADDITIONAL OBSERVATIONS
Basal medium	Traditionally described basal media formulations consist of DMEM, F-12, M199, RPMI 1640, Iscove's modification IMDM and combinations Thereof.	We recommend the use of "rich" basal media formulation such as DMEM/F12, Williams medium E or Ham's F12.	It has been reported that the amino acids Gln, Arg, His, Leu, Thr, Ile, Lys, Ser, Tyr, Phe, Val and Met were consumed, and 4 amino acids (Glu, Asp, Ala and Pro) were produced during the growth of Vero cells in batch culture. Leu, Thr, Ile, Lys, Phe and Tyr were consumed quickly only in the exponential phase of growth and consumed very slowly in the stationary phase of growth. GAO Hong-liang, CONG Wei, OUYANG Fan. Amino Acid Metabolism of Vero Cells in Batch Culture. Chinese Journal of Process Engineering, 2001,1(2): 176-179.
XerumFree™ XF212 concentration of use	XerumFree XF212 is typically used at the same percentage as FBS, i.e. 10% for most cell lines, including Vero, CHO, BHK-21.	For Vero adherent or suspension cultures suggested use is 10% XerumFree™ + 90% basal media.	Use of higher concentrations (up to 15% XerumFree XF212) during the production phase may prove beneficial due to the additional nutrient requirements during the assembly, morphogenesis, budding and release of progeny virus particles.
Additional upstream growth requirements	In our experience XerumFree™ sustains consistent growth and proliferation of Vero cells without addition of growth factors or hormones.	Addition of recombinant insulin (1.25 mg/liter) and EGF (12.5 µg/liter) can stimulate growth of Vero batch cultures.	During the stationary phase, use of dexamethasone (10 ⁻⁶ M) can compensate for the absence of corticosteroids from FBS. These hormones stimulate overall protein synthesis, including those of viral origin. However, at TNC BIO we noted a negative impact on the cell growth by dexamethasone when employed during the exponential growth phase.
Application of the ACGS principle	Vero cells, like most other cell types are subject to autocrine growth factor signalling. Analysis of growth media suggest the release by Vero cells of a number of growth factors, including TGF-β, PDGF, IL-6, LIF, and also a set of proteins from the Insulin-like Growth Factor Binding Protein superfamily.	Autocrine growth factors are absent in the fresh culture medium at the seeding stage. The complementation with conditioned medium derived from earlier high cell density serum-free cultures compensates for the absence of autocrine growth factors. This can be done by adding spent medium from confluent serumfree cultures (25-50% ratio) to the seeding suspension.	Autocrine cell growth supplementation stimulates cell attachment. Spreading and proliferation. Please contact TNC BIO's technical service for more detailed information for the detailed technical note regarding autocrine cell growth. (welcome@tncbio.com or tech@tncbio.com)

Please contact TNC BIO's technical service for more detailed information (tech@tncbio.com). See also *TNC BIO technical note on cell attachment and ACGS*.