

Explore Performances of the EX-CELL® CD Insect Cell Medium

A chemically defined medium specialty formulated to get the best performances for *Spodoptera frugiperda* (Sf) cells



Sandy McNorton¹, Thibaut Deschamps¹, Nathan Stegemann¹, Kim Schrag¹, Kate Achtien² and Charlotte Javalet³
1: **MilliporeSigma**, Lenexa, KS, 66215, USA, 2: **MilliporeSigma**, St. Louis, MO, USA, 3: **Merck**, 38240 Meylan, France

Introduction

The Baculovirus Expression Vector System (BEVS) is a powerful eucaryotic vector system used to produce viral vaccines and gene therapy vectors. *Spodoptera frugiperda* (Sf) cell lines are widely used as hosts for BEVS. However, FDA's Retrovirus Laboratory discovered that the majority of Sf cells (such as Sf9) contain an unknown Sf-rhabdovirus that is now considered a process contaminant and must be eliminated during the bioprocessing (Hailun Ma *et al.* 2014). Because viral safety is essential in the manufacture of biopharmaceuticals and required to ensure patient safety, we offer a proven Sf9-rhabdovirus-negative (Sf-RVN®) Insect Cell Line that improves the safety profile of our customers' bioprocesses. In order to get excellent growth and productivity of the Sf-RVN® Insect Cell Line, we specifically developed a chemically defined medium: the EX-CELL® CD Insect Cell Medium. Combined, these two products form the Sf-RVN® Platform and provide a high performant rhabdovirus-free BEVS alternative to produce recombinant proteins, Virus-Like Particles (VLP) for viral vaccines, and Adeno-Associated Viruses (AAV) to treat genetic diseases.

In this study, we explored the performances of the EX-CELL® CD Insect Cell Medium. We compared the growth of two *Spodoptera frugiperda* (Sf) cells, our rhabdovirus-free Sf-RVN® Insect Cell Line and its parent, the Sf9 cell line, both cultivated in 6 different cell culture media. Two of them are chemically defined (one is the EX-CELL® CD Insect Cell Medium and the other a competitor media). The 4 others are not chemically defined and contains hydrolysates (one of them is our EX-CELL® 420 Serum-Free Medium, the others are competitor media). We found that the EX-CELL® CD Insect Cell Medium outperforms all media tested, including the not chemically defined media, for both Sf-RVN® and Sf9 cells. Then, in the same conditions, we evaluated the protein productivity by infecting cells with a baculovirus encoded for the secreted alkaline phosphatase (SEAP). For the Sf-RVN® Insect Cell Line, our data shown that the EX-CELL® CD Insect Cell Medium enables the second-best production after the EX-CELL® 420 Serum-Free Medium while improving lot-to-lot consistency of the medium, which is a common issue with hydrolysate containing formulations. In addition, by comparing the productivity of the two Sf cells, we demonstrated that the Sf-RVN® Platform had the highest SEAP productivity.



Methods

Media preparation:

EX-CELL® CD Insect Cell Medium, liquid media (Cat n°14380C, Merck) was supplemented with 3 mL/L of SyntheChol® and dry powder medium (Cat no. 24381C, Merck) was hydrated per the label.

Competitor media was purchased and used as a liquid medium.

Growth promotion assay

Sf-RVN® and Sf9 cells were adapted in the EX-CELL® CD Insect Cell Medium for at least 5 passages prior to the growth curves. Cells were seeded at 0.5x10⁶ cells/mL on day 0, viable cells density and viability were followed for 7 days. Cell counts were performed on a ViCell XR.

The same process for adaptation and growth of the Sf-RVN® cells in different media was followed.

Recombinant protein production by BEVS

Recombinant baculovirus producing secreted alkaline phosphatase (SEAP) was produced in Sf-RVN® cells and titer was assessed by plaque assay. Cells were seeded at 2x10⁶ cells/mL and exposed to recombinant baculovirus at an multiplicity of infection (MOI) of 1. Cells were harvested on day 2 and 3 and SEAP production was measured with a reporter gene chemiluminescent detection system on a TECAN plaque reader.

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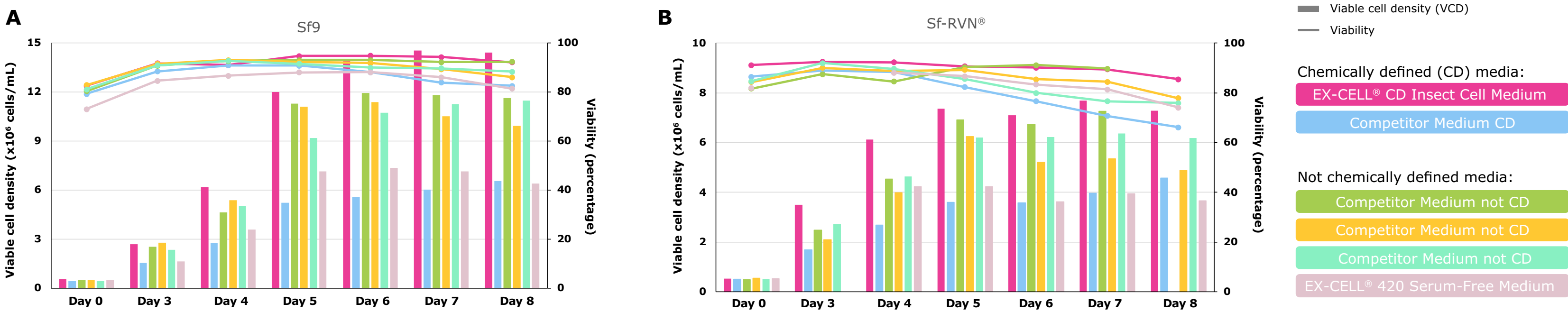


Figure 1: EX-CELL® CD Insect Cell Medium is the best medium to support Sf-RVN® (A) and Sf9 (B) cells growth. Cells were adapted for at least 5 passages in 6 different cell culture media. Two of them are chemically defined (one is the EX-CELL® CD Insect Cell Medium and the other a competitor media). The 4 others are not chemically defined and contains hydrolysates (one of them is our EX-CELL® 420 Serum-Free Medium, the others are competitor media). After adaptation, cells were seeded 0.5x10⁶ cells/mL on day 0. Viable cell density (VCD) and viability were followed for 7 days.

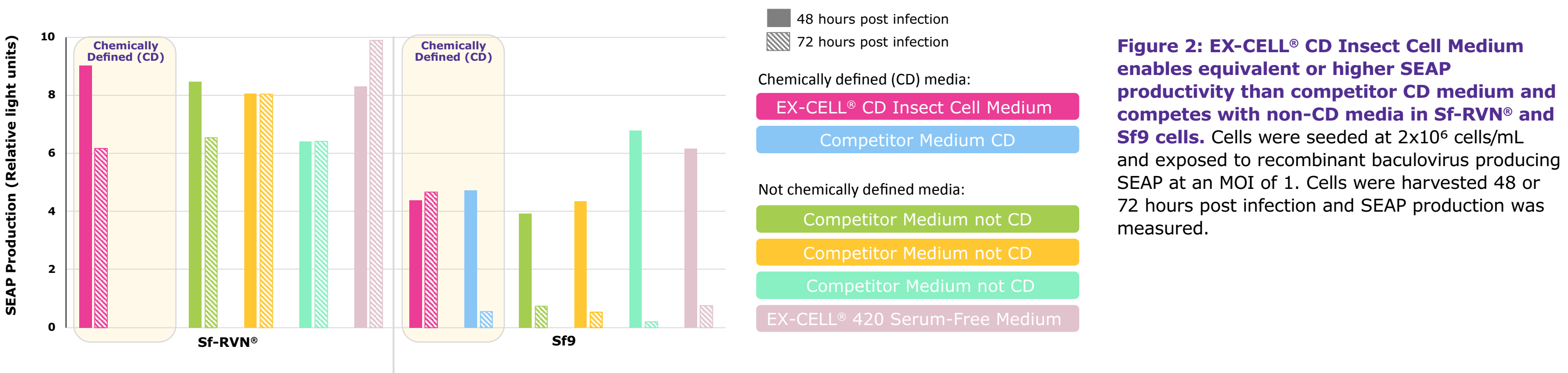


Figure 2: EX-CELL® CD Insect Cell Medium enables equivalent or higher SEAP productivity than competitor CD medium and competes with non-CD media in Sf-RVN® and Sf9 cells. Cells were seeded at 2x10⁶ cells/mL and exposed to recombinant baculovirus producing SEAP at an MOI of 1. Cells were harvested 48 or 72 hours post infection and SEAP production was measured.

Conclusions

- The EX-CELL® CD Insect Cell Medium has been formulated to optimize the growth, viability and productivity of the Sf-rhabdovirus-negative (Sf-RVN®) Insect Cell Line. Combined, these two products form the Sf-RVN® Platform and provide a high performant rhabdovirus-free BEVS alternative to produce recombinant proteins, Virus-Like Particles (VLP) for viral vaccines, and Adeno-Associated Viruses (AAV) to treat genetic diseases.
- In this study we demonstrated that the EX-CELL® CD Insect Cell Medium outperforms all media tested, including the not chemically defined media, for the growth of both Sf-RVN® and Sf9 cells.
- For the Sf-RVN® Insect Cell Line, our data shown that the EX-CELL® CD Insect Cell Medium enables the second-best production after the EX-CELL® 420 Serum-Free Medium while improving lot-to-lot consistency of the medium, which is a common issue with hydrolysate containing formulations.
- By comparing the productivity of the two Sf9 cells, we demonstrated that the Sf-RVN® cells had a higher SEAP productivity than the Sf9 cells, especially when cultivated with companion EX-CELL® CD Insect Cell Medium.



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