

# 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 McNorton<sup>1</sup>, Thibaut Deschamps<sup>1</sup>, Nathan Stegemann<sup>1</sup>, Kim Schrag<sup>1</sup>, Kate Achtien<sup>2</sup> and Charlotte Javalet<sup>3</sup>

1: MilliporeSigma, Lenexa, KS, 66215, USA, 2: MilliporeSigma, St. Louis, MO, USA, 3: Merck KGaA, Darmstadt Germany, 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 out-performs 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, MilliporeSigma) was supplemented with 3 mL/L of SyntheChol® and dry powder medium (Cat no. 24381C, MilliporeSigma) 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 0.5x10<sup>6</sup> 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.

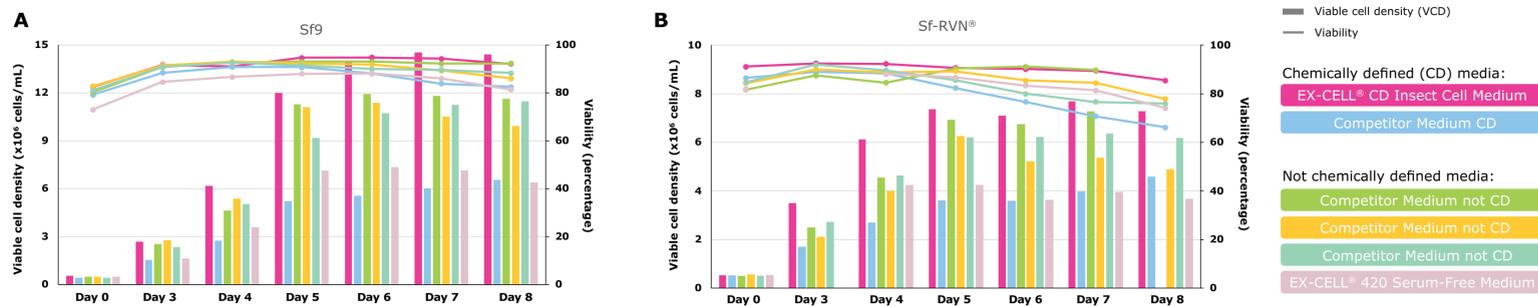
### Recombinant protein production by BEVS

Recombinant baculovirus producing secreted alkaline phosphatase (SEAP) was produced in Sf-RVN® cells and titer was assessed by plaque assay. Cells were seeded at 2x10<sup>6</sup> cells/mL and exposed to recombinant baculovirus at a 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.

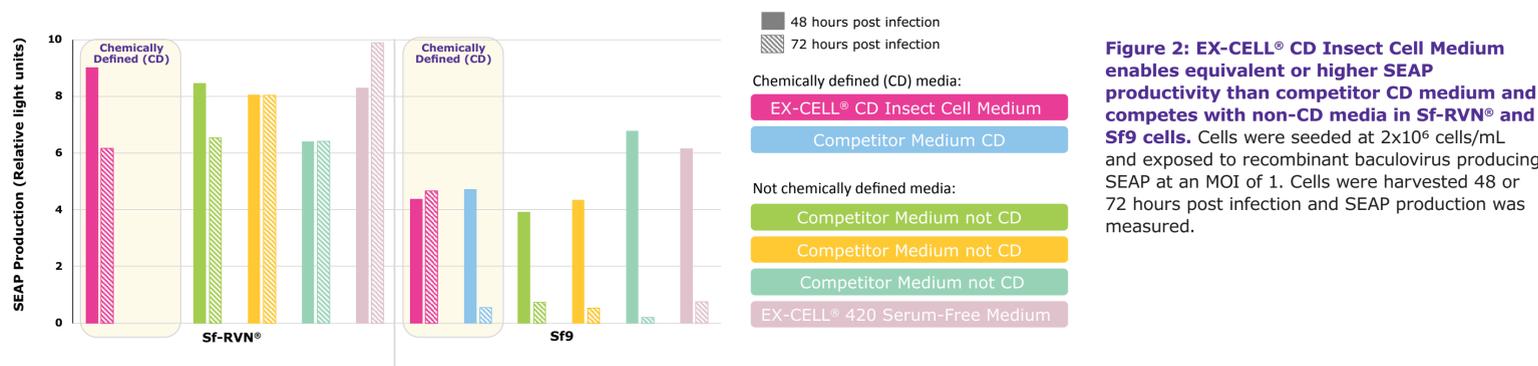
MilliporeSigma is the U.S. and Canada Life Science business of Merck KGaA, Darmstadt, Germany.

EMDMillipore.com

© 2022 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved. MilliporeSigma, SAFC, the vibrant M, Sf-RVN, SyntheChol, and EX-CELL are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources. Lit. No. MS\_P091035X ver 1.0



**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 0.5x10<sup>6</sup> 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 2x10<sup>6</sup> 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 out-performs 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.



## Results/Discussion

### Cell growth assay

We performed a cell growth assay to evaluate the performance of the EX-CELL® CD Insect Cell Medium to support the growth of two *Spodoptera frugiperda* cell lines. The first one is our Sf-RVN® Insect Cell Line, a proven Sf9 rhabdovirus-free cell line, the other a Sf9 cell line. These cells were adapted and 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). As shown in **Figure 1**, we found that the EX-CELL® CD Insect Cell Medium out-performs all media tested, including the not chemically defined media, for both Sf-RVN® and Sf9 cells. This result demonstrates that the EX-CELL® CD Insect Cell Medium is well optimized for the growth of the Sf-RVN® Insect Cell Line but also that this medium is the best medium to support growth of other Sf9 cells. In addition, this chemically defined medium improves lot-to-lot consistency, which is a common issue with hydrolysate containing formulations.

### SEAP Productivity

We used the secreted alkaline phosphatase (SEAP) to evaluate the performance of the EX-CELL® CD Insect Cell Medium to support the protein productivity of the Sf-RVN® and Sf9 cells. Cells were adapted and cultivated as previously described. Then cells were infected by a recombinant baculovirus producing SEAP and the SEAP productivity were measured 48 and 72 hours post infection. As shown in **Figure 2**, for Sf-RVN® cells, we found that the competitor CD medium didn't support the production of the SEAP whereas the EX-CELL® CD Insect Cell Medium enabled the second-best production after our EX-CELL® 420 Serum-Free. However, because this media contains hydrolysate, it can meet lot-to-lot consistency issues. This result proves that the EX-CELL® CD Insect Cell Medium has been optimized to get the best protein productivity of the Sf-RVN® Insect Cell Line. Regarding the Sf9 cells, we found that the EX-CELL® CD Insect Cell Medium is comparable with the competitor CD medium, both being equivalent or lower than not CD media. In addition, when we compared the protein productivity of each cell line, our experiments demonstrated that the Sf-RVN® Insect Cell Line had a higher SEAP productivity than the Sf9 cells, especially when cultivated with companion EX-CELL® CD Insect Cell Medium.

**SAFC®**  
Pharma & Biopharma Raw  
Material Solutions