# Novel Sf9 rhabdovirus-negative cell line and chemically defined media platform

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The life science business of Merck KGaA Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

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Agenda

# **Upstream Production Systems for Vaccines:** Insect cell expression systems



# **Sf-RVN® Platform:** Improving the Safety Profile of Baculovirus-Insect Cell Bioprocess



# **Sf-RVN® Platform Performances**



# 01 Upstream Production Systems for vaccines: Insect cell expression systems

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# **Insect Cell Expression Systems** History and characteristics of Sf cell lines

#### History

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**IPLB-Sf21AE** is the first Sf cell line isolated by Vaughn *et al.* from a pupal ovaries at the USDA Insect Pathology Laboratory in Maryland, USA, commonly known as **Sf21** 

# 1977

**Sf9** is a subclone of **Sf21**, widely used for research purposes.

1987

#### 1983 First description of the Baculovirus Expression Vector System (BEVS)

by Smith et al., production of IFN-β using a polyhedrin promoter

#### Versatility of the insect cell. **BEVS became widespread** and is one of the most common platforms for recombinant protein expression

19905

Spodoptera frugiperda (Sf) cells

# X

#### Easy to cultivate

- Attached or suspension cell culture
- No CO<sub>2</sub> requirement
- Grow at low temperature (27°C)
- Easily scaled

### Safety

Resistant to mammalian viruses

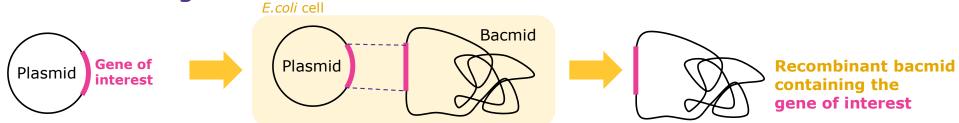
Sf cell lines are widely used as hosts for BEVS, a powerful eucaryotic vector system used to produce recombinant proteins, viral vaccines and gene therapy vectors



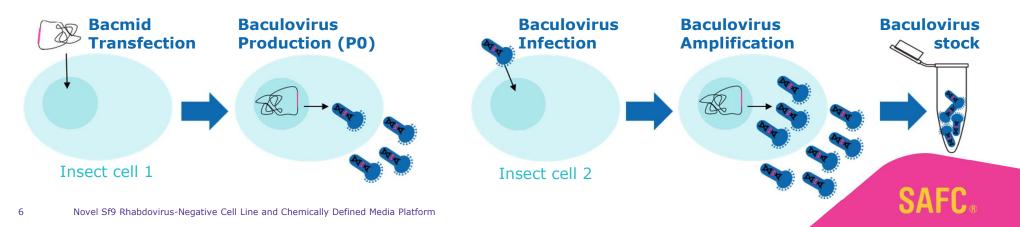
# **Baculovirus Expression Vector System (BEVS)**

A powerful eucaryotic vector system

# **1** Bacmid cloning



# **2** Baculovirus stock production

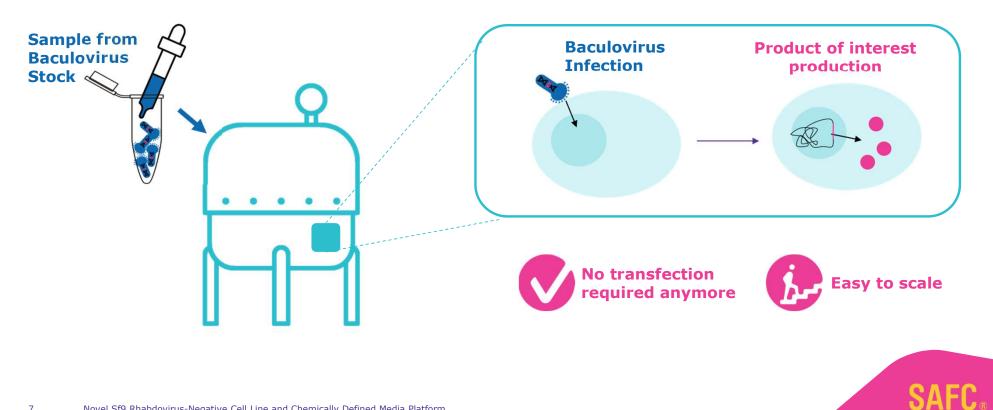


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### **Baculovirus Expression Vector System (BEVS)**

A powerful eucaryotic vector system

### **3 Product of interest production**



### **Introduction to Insect Cell Expression Systems** Approved therapeutics produced with insect cells

Component	Clinical Indication	Product Type	Manufacturer
For humans			
Flublok <sup>®</sup> vaccine	Influenza	Subunit	Sanofi Pasteur
Cervarix <sup>®</sup> vaccine	Human Papillomavirus	VLP	GSK
Provenge <sup>®</sup> immunotherapy	Prostate Cancer	Immunotherapy	Dendreon
Glybera <sup>®</sup> gene therapy treatment*	Lipoprotein lipase deficiency	rAAV	uniQure
For animals			
Porcilis <sup>®</sup> Pesti vaccine	Classical swine fever	subunit	MSD Animal Health
Circumvent <sup>®</sup> PCV vaccine	Porcine circovirus type 2	VLP	MSD Animal Health
Ingelvac CircoFLEX® vaccine	Porcine circovirus type 2	VLP	Boehringer Ingelheim
Porcilis <sup>®</sup> PCV vaccine	Porcine circovirus type 2	VLP	MSD Animal Health

#### Sf9 has been used for several approved therapeutics including production of viral vectors, vaccines and recombinant proteins

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# The Discovery of the FDA's Retrovirus Laboratory Sf9 cells are contaminated with a novel rhabdovirus



Methods: Sequencing (NGS), RT-PCR and Electronic Microscopy (TEM)



Phylogeny: The previously unknown rhabdovirus, was found to be more closely related to plant rhabdovirus than to invertebrate or vertebrate rhabdovirus

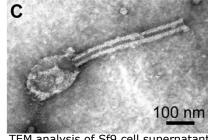


Virus load: rhabdovirus is **constitutively produced** by Sf9 –level of viral particles estimated at 2 x 10<sup>9</sup> particles per mL



Persistent infection: Sf-rhabdovirus detected in original vials of Sf9 and of the parent Sf21 cells





TEM analysis of Sf9 cell supernatant





# Viral safety is essential in the manufacturing of biopharmaceuticals and required to ensure

# **patient Safety**





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A robust biosafety approach built upon the pillars of:

"prevent, detect, remove"

It encompasses use of high-quality raw materials to prevent introduction of viruses

#### To enhance risk mitigation, we offer a Sf-rhabdovirus negative (Sf-RVN®) cell line

# 2 Sf-RVN<sup>®</sup> Platform: Improving the Safety Profile of Baculovirus-Insect Cell Bioprocess

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# **Risk Assessment and Mitigation in BioProcessing**

### Process economics High titer Exclusion of unnecessary additives Concentrated feeds High growth rate and cell densities Culture longevity Reduce product and process impurities

Business continuity Regulatory and IP compliance Reliable supply chain

13 Novel Sf9 Rhabdovirus-Negative Cell Line and Chemically Defined Media Platform

#### Quality risk management approach

Reduce regulatory and risk considerations

Reduced analytical workload Product stability through harvest Clone stability Product homogeneity

#### Speed to market

Fast cell line development process Screen molecules earlier in discovery process

Maintain/enable favorable product quality attributes

Scalable process



# **Sf-RVN® Platform** Description

**SAFC**<sup>®</sup>



Novel Sf9 Rhabdovirus-Negative Cell Line and Chemically Defined Media Platform

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# Sf-Rhabdovirus-Negative (Sf-RVN®) Cell Line Enhanced risk mitigation of baculovirus-insect cell bioprocess

### **Cell Line Development**

The Sf-RVN® Insect Cell Line has been developed and characterized by the Dr Don Jarvis group and Glycobac LLC *Exclusive licensing with MilliporeSigma for bioproduction* 

### Sf-RVN® GMP Banked

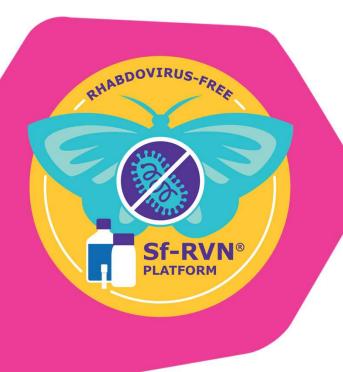
- A proven Sf9 rhabdovirus-free cell line\*
- Same characteristics as Sf9 cells\*
- GMP banked and adventitious agent tested cell line
- Full traceability and documentation for regulatory filings
- Technical user guide with detailed protocols for optimal performances

\*Maghodia et al. 2016 and 2017

RHABDOVIRUS-A

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# **EX-CELL® CD Insect Cell Media** Optimized for the Sf-RVN® Insect Cell Line



#### The need

A chemically defined medium specifically optimized for Sf-RVN® Insect Cell Line to support excellent growth and productivity

#### **Our solution**

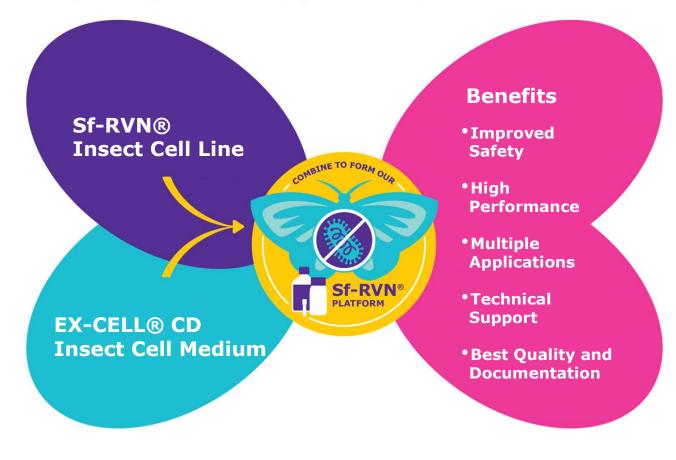
- EX-CELL® CD Insect Cell Medium
- Optimized for the Sf-RVN® Insect Cell Line
- Supports growth of multiple insect cell lines (Sf21, Sf9, Sf-RVN®, S2, Tni and C636 cells)
- Chemically defined and animal component free
- · Available in liquid and dry powdered media





### **Sf-RVN® Platform**

For improving the safety profile of your baculovirus-insect cell bioprocess



#### **Benefits**

• Improved Safety Enhanced risk mitigation with the rhabdovirus negative cell line

• High Performance

Optimized to get low doubling time, high cell viability and excellent productivity

• Multiple Applications

Optimal to produce recombinant proteins, viral like particles (VLP) and adeno-associated virus (AAV)

- Technical Support
   Technical user guide with detailed
   protocols for optimal performances
- Best Quality & Documentation Regulatory support and quality documentation

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# **O3** Sf-RVN<sup>®</sup> Platform performances

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# **EX-CELL® CD Insect Cell Medium** Supports the growth of multiple insect cells

#### 6 Insect cell lines / 1 Medium



EX-CELL® CD Insect Cell Medium

#### **Experimental Parameters**

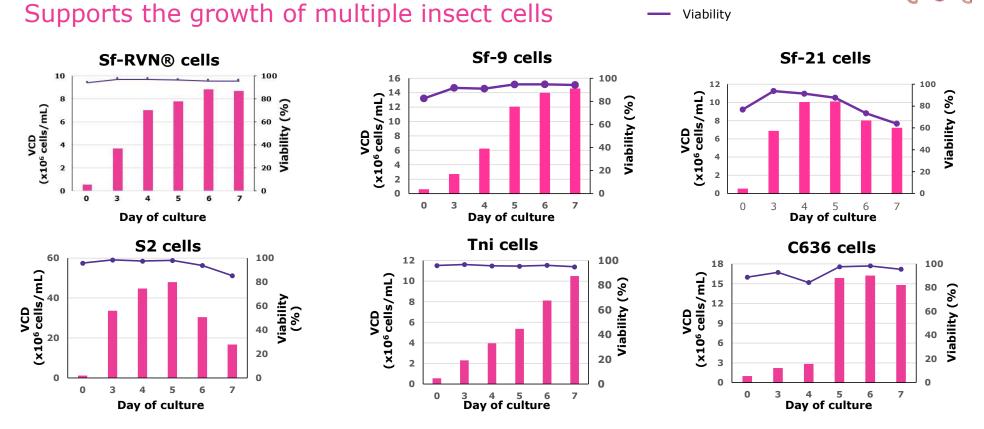
Cells adapted in the medium for at least 5 passages

#### **Cell growth assay**

- Viable Cell Density (VCD)
- Viability (%)
- Measured on days 0, 3, 4, 5, 6 and 7







Viable cell density (VCD)

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Robust growth of multiple insect cells with the EX-CELL® CD Insect Cell Medium

**EX-CELL® CD Insect Cell Medium** 

# **EX-CELL® CD Insect Cell Medium**

The best medium to support growth of Sf9 and Sf-RVN® insect cell lines

#### 2 Insect cell lines / 6 Media



Sf9 cells



Sf-RVN® cells

Chemically defined (CD) media: EX-CELL<sup>®</sup> CD Insect Cell Medium Competitor Medium CD

#### Non-chemically defined media:

Competitor Medium non-CD Competitor Medium non-CD Competitor Medium non-CD

#### **Experimental Parameters**

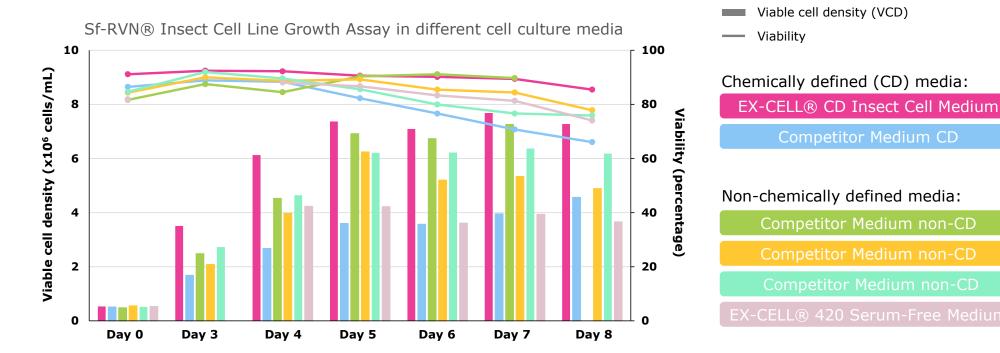
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#### **Cell growth assay**

- Viable Cell Density (VCD)
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- Measured at days 0, 3, 4, 5, 6 and 7



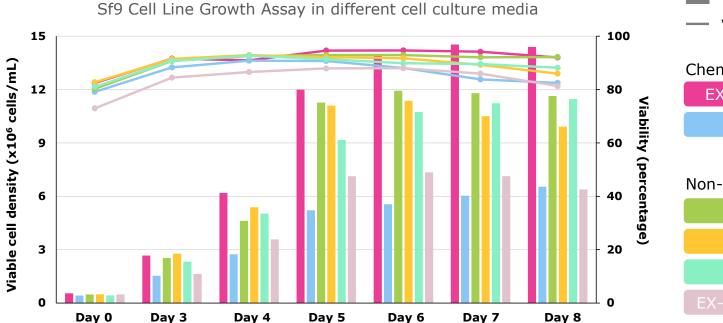


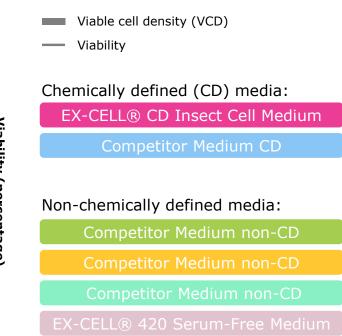


**EX-CELL® CD Insect Cell Medium outperforms competitor CD medium as well as other non-CD media for supporting the Sf-RVN® cell growth** 



# **EX-CELL® CD Insect Cell Medium** The best medium to support growth of Sf9 cell line





**EX-CELL® CD Insect Cell Medium outperforms competitor CD medium as well as other non-CD media for supporting the Sf9 cell growth** 

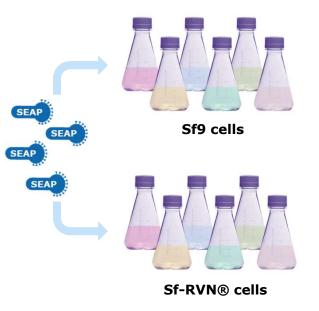


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# **EX-CELL® CD Insect Cell Medium** Enables high protein productivity of the Sf cell lines

### 2 Insect cell lines / 6 Media / 1 Baculovirus



# Chemically defined (CD) media:

EX-CELL® CD Insect Cell Medium

Competitor Medium CD

#### Non-chemically defined media:

Competitor Medium non-CD

Competitor Medium non-CD

Competitor Medium non-CD

EX-CELL® 420 Serum-Free Medium

#### **SEAP**

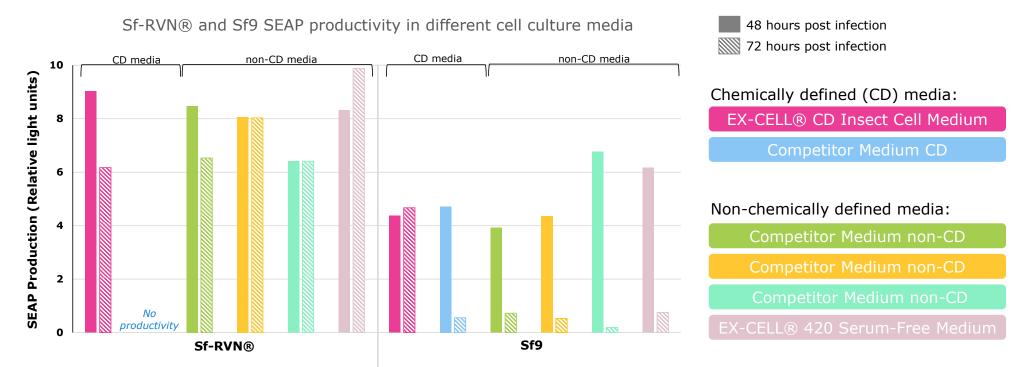
- Enzyme (secreted alkaline phosphatase)
- Recombinant secreted protein ~64kDa
- Reporter gene chemiluminescent detection system

#### **Experimental Parameters**

- Cells adapted in media for a least 5 passages
- Cells seeded at 2x10<sup>6</sup> cells/mL
- Baculovirus infection at MOI 1
- SEAP productivity measured at 48 and 72 hours post infection

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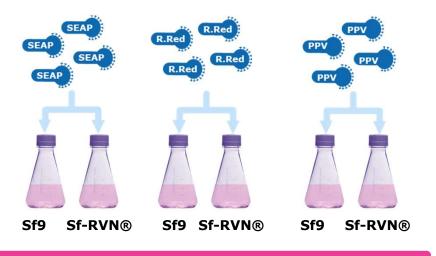
# **EX-CELL® CD Insect Cell Medium** Enables high protein productivity of the Sf cell lines



EX-CELL® CD Insect Cell Medium enables equivalent or higher protein productivity than competitor CD medium and competes with non-CD media in Sf-RVN® and Sf9 cells

# **EX-CELL® CD Insect Cell Medium** Sf-RVN® vs Sf9 productivity

#### 2 Insect cell lines / 1 Media / 3 Baculoviruses



EX-CELL® CD Insect Cell Medium



#### **SEAP (Secreted alkaline phosphatase)**

#### **Rudolph Red**

- Red Fluorescent Protein, florescent measured
- Intracellular, 185 kDa

#### **PPV (Porcine Parvovirus)**

- Small non-enveloped virus
- Spherical shell capsid (~ 28 nm in diameter)
- Cell lysed; capsid protein measured

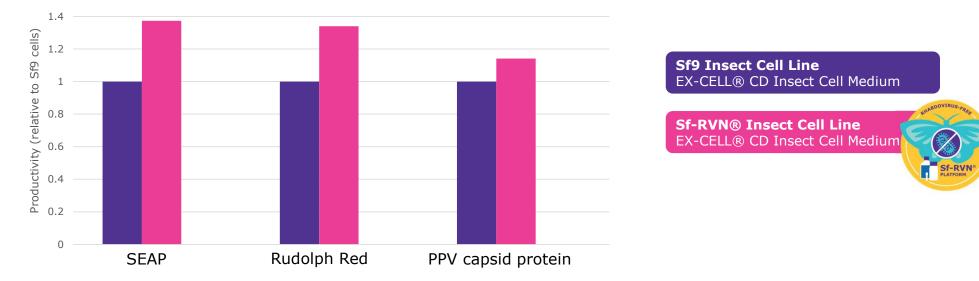
#### **Experimental Parameters**

- Cells adapted in medium for a least 5 passages
- Cells seeded at 2x10<sup>6</sup> cells/mL
- Baculovirus infection at MOI 0.1
- Measured at 72 hours post infection



# **Sf-RVN® Platform** Provides a better protein productivity than Sf9 cells

Sf-RVN® vs Sf9 productivity in EX-CELL® CD Insect Cell Medium

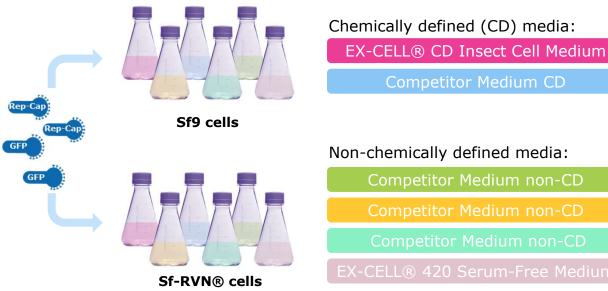


# Higher protein productivity of the Sf-RVN® Insect Cell Line than Sf9 cells, both cultivated with the EX-CELL® CD Insect Cell Medium



# EX-CELL® CD Insect Cell Medium Enables high AAV2 titer of Sf cell lines

### 2 Insect cell lines / 6 Media / 2 Baculoviruses





#### **AAV (Adeno-Associated Virus)**

- AAV2 serotype
- Recombinant intracellular AAV2

#### 2 Baculovirus system

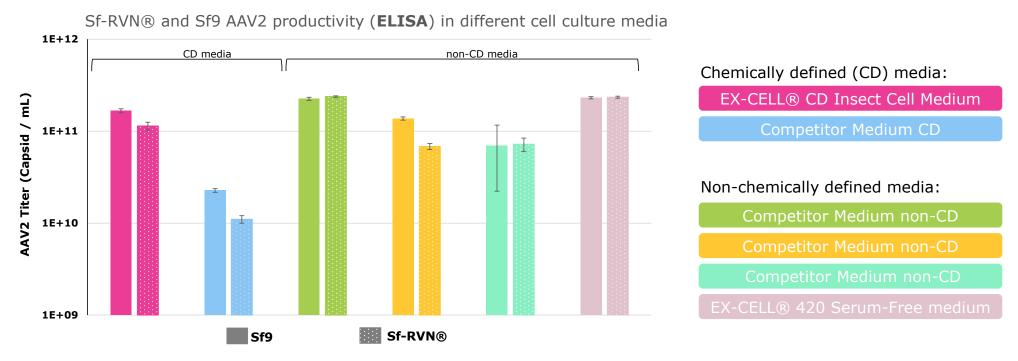
- Combined Rep-Cap in one baculovirus
- Transgene baculovirus (encoded for GFP)
- Co-infection

#### **Experimental Parameters**

- Cells adapted in medium for a least 5 passages
- Cells seeded at 2x10<sup>6</sup> cells/mL
- Baculoviruses infection at MOI 0.01
- Measured at 96 hours post infection
- Total capsid measured by ELISA assay
- Full capsid measured by ddPCR



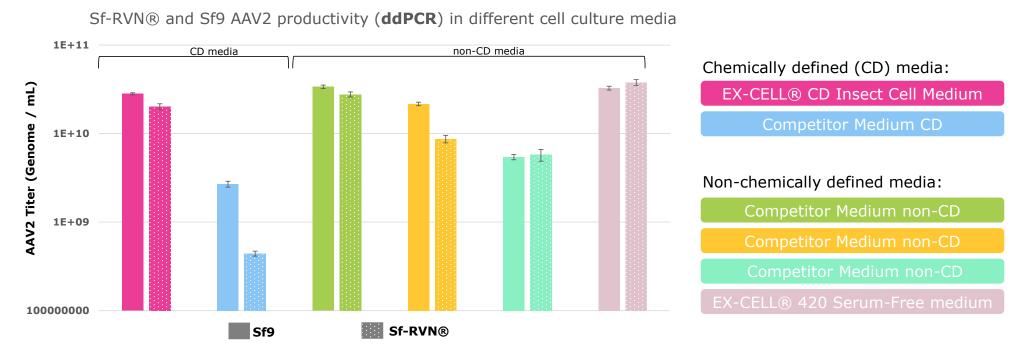
# **EX-CELL® CD Insect Cell Medium** Enables High AAV2 titer



EX-CELL® CD Insect Cell Medium outperforms competitor CD medium and competes with non-CD media for AAV2 production in Sf-RVN® and Sf9 cells The Sf-RVN® Platforms produces ~1x10<sup>11</sup> capsid/mL (total capsid)



# **EX-CELL® CD Insect Cell Medium** Enables High AAV2 titer



EX-CELL® CD Insect Cell Medium outperforms competitor CD medium and competes with non-CD media for AAV2 production in Sf-RVN® and Sf9 cells The Sf-RVN® Platforms produces ~2x10<sup>10</sup> genome/mL (full capsid)

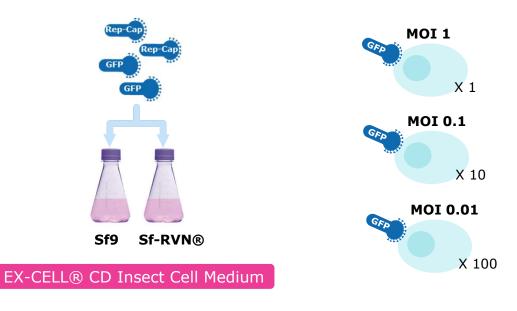
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# **Sf-RVN® Platform** Enables High AAV2 titer

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#### 2 Insect cell lines / 1 Medium / 2 Baculoviruses



#### **AAV (Adeno-Associated Virus)**

- AAV2 serotype
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#### 2 Baculovirus system

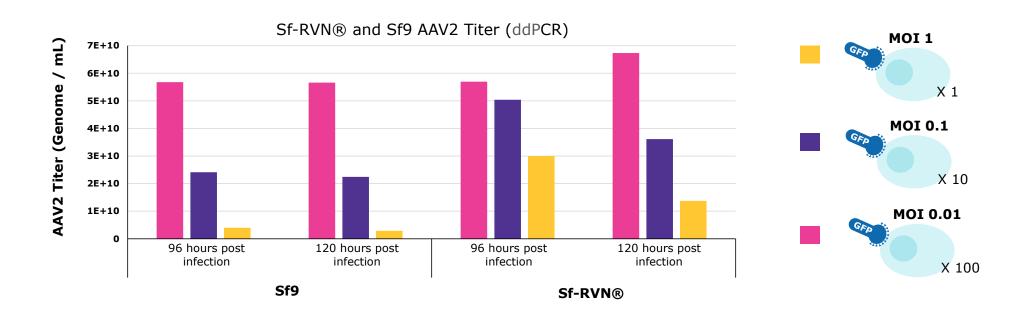
- Combined Rep-Cap in one baculovirus
- Transgene baculovirus (encoded for GFP)
- Co-infection

#### **Experimental Parameters**

- Cells adapted in medium for a least 5 passages
- Cells seeded at 2x10<sup>6</sup> cells/mL
- 3 MOI (multiplicity of infection): 0.01, 0.1 and 1
- Full capsid (genome concentration) measured by ddPCR
- Measured at 96 and 120 hours post infection



# **Sf-RVN® Platform** Enables High AAV2 titer



High full AAV2 titer ( $\sim 6 \times 10^{10}$  genome/mL) produced by the two Sf cell lines, better production with the lowest MOI (0.01)

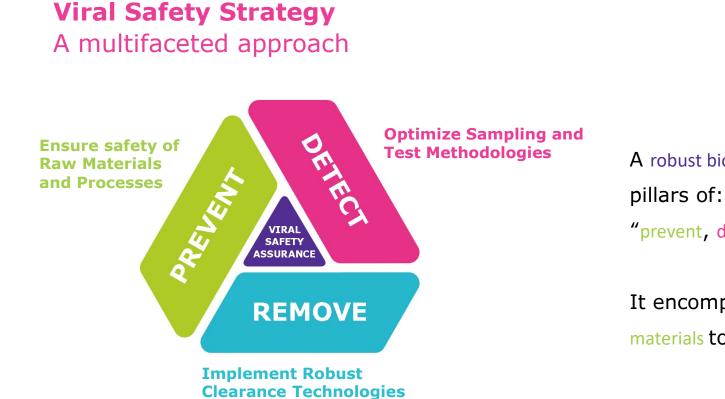
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# 04 Summary

# **SAFC**®

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A robust biosafety approach built upon the pillars of:

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It encompasses use of high-quality raw materials to prevent introduction of viruses

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# **Risk Assessment and Mitigation in BioProcessing**

### **Process economics** High titer Exclusion of unnecessary additives Concentrated feeds High growth rate and cell densities Culture longevity Reduce product and process impurities

**Business continuity** Regulatory and IP compliance Reliable supply chain

Novel Sf9 Rhabdovirus-Negative Cell Line and Chemically Defined Media Platform

#### Quality risk management approach

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#### Speed to market

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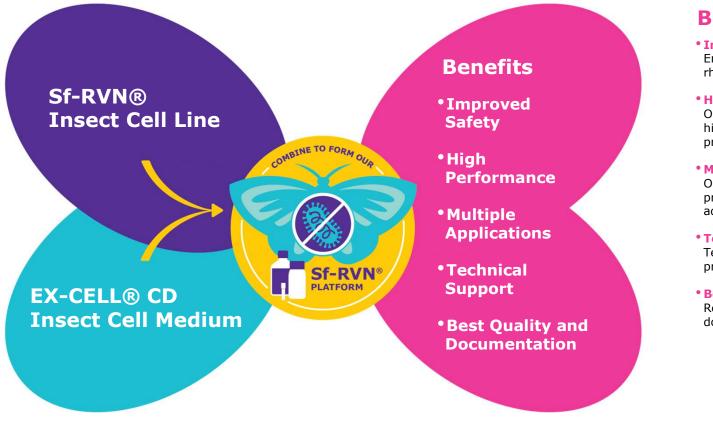
Maintain/enable favorable product quality attributes

Scalable process



### **Sf-RVN® Platform**

For improving the safety profile of your baculovirus-insect cell bioprocess



#### **Benefits**

• **Improved Safety** Enhanced risk mitigation with the rhabdovirus negative cell line

• High Performance

Optimized to get low doubling time, high cell viability and excellent productivity

• Multiple Applications Optimal to produce recombinant proteins, viral like particles (VLP) and

adeno-associated virus (AAV)

- Technical Support Technical user guide with detailed protocols for optimal performances
- Best Quality & Documentation Regulatory support and quality documentation

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