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.
Agenda

1. Upstream Production Systems for Vaccines: Insect cell expression systems
2. Sf-RVN® Platform: Improving the Safety Profile of Baculovirus-Insect Cell Bioprocess
3. Sf-RVN® Platform Performances

SAFC®
Pharma & Biopharma Raw Material Solutions
Upstream Production Systems for vaccines: Insect cell expression systems
Insect Cell Expression Systems
History and characteristics of Sf cell lines

History

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

1977

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

1983

First description of the Baculovirus Expression Vector System (BEVS) by Smith et al., production of IFN-β using a polyhedrin promoter.

1987

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

1990s

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.

Spodoptera frugiperta (Sf) cells

Easy to cultivate

- Attached or suspension cell culture
- No CO₂ requirement
- Grow at low temperature (27°C)
- Easily scaled

Safety

- Resistant to mammalian viruses
Baculovirus Expression Vector System (BEVS)
A powerful eucaryotic vector system

1 Bacmid cloning

2 Baculovirus stock production
Baculovirus Expression Vector System (BEVS)
A powerful eucaryotic vector system

3 Product of interest production

Sample from Baculovirus Stock

Baculovirus Infection

Product of interest production

No transfection required anymore

Easy to scale
### Introduction to Insect Cell Expression Systems

Approved therapeutics produced with insect cells

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

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

*Marketing authorization not renewed*
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 10^9 particles per mL

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

This novel Sf-rhabdovirus is not considered harmful to humans but considered as an adventitious agent and removal from bioprocesses must be proven
Viral safety is essential in the manufacturing of biopharmaceuticals and required to ensure patient Safety.
To enhance risk mitigation, we offer a Sf-rhabdovirus negative (Sf-RVN®) cell line.
Sf-RVN® Platform: Improving the Safety Profile of Baculovirus-Insect Cell Bioprocess
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

**Quality risk management approach**
- Reduce regulatory and risk considerations
- Reduced analytical workload
- Product stability through harvest
- Clone stability
- Product homogeneity

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

**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

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

**Sf-RVN® Insect Cell Line**
**EX-CELL® CD Insect Cell Medium**
Sf-RVN® Platform performances
EX-CELL® CD Insect Cell Medium
Supports the growth of multiple insect cells

6 Insect cell lines / 1 Medium

3 Spodoptera frugiperda cell lines
Sf-RVN®  Sf9  Sf21

3 other insect cell lines
S2  Tni  C636

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

Robust growth of multiple insect cells with the 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

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

Experimental Parameters
- Cells adapted in the medium for at least 5 passages

Cell growth assay
- Viable Cell Density (VCD)
- Viability (%)
- Measured at days 0, 3, 4, 5, 6 and 7
EX-CELL® CD Insect Cell Medium
The best medium to support growth of Sf-RVN® Insect Cell Line

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.

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
- EX-CELL® 420 Serum-Free Medium
**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 at least 5 passages
- Cells seeded at 2x10^6 cells/mL
- Baculovirus infection at MOI 1
- SEAP productivity measured at 48 and 72 hours post infection
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

SEAP (Secreted alkaline phosphatase)

Rudolph Red
- Red Fluorescent Protein, fluorescent 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 2x10^6 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

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

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

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 at least 5 passages
- Cells seeded at $2 \times 10^6$ 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

Sf-RVN® and Sf9 AAV2 productivity (ELISA) in different cell culture media

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 \(1 \times 10^{11}\) capsid/mL (total capsid).
EX-CELL® CD Insect Cell Medium
Enables High AAV2 titer

Sf-RVN® and Sf9 AAV2 productivity (ddPCR) in different cell culture media

Chemically defined (CD) media:
- EX-CELL® CD Insect Cell Medium
- Competitor Medium CD

Non-chemically defined media:
- Competitor Medium non-CD (three instances)
- EX-CELL® 420 Serum-Free medium

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^{10} genome/mL (full capsid)
Sf-RVN® Platform
Enables High AAV2 titer

2 Insect cell lines / 1 Medium / 2 Baculoviruses

EX-CELL® CD Insect Cell Medium

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 at least 5 passages
• Cells seeded at 2x10^6 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 (~6x10^{10} genome/mL) produced by the two Sf cell lines, better production with the lowest MOI (0.01)
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.
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Insect Cell Line

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