



# Sapphire™ Baculovirus DNA and Transfection Kit

## PRODUCT SUMMARY

<b>Cat. No:</b>	<b>BVD-10002A</b>
<b>Component:</b>	1 vial of 5 ug Sapphire™ Baculovirus DNA 1 vial of DNA Shuttle Transfection Reagent TNM-FH Insect Medium with 10 % FBS, 1 L
<b>Storage:</b>	Store at 4°C. Never freeze the viral DNA.
<b>Stability:</b>	All components are stable for 6 months when stored properly.

## DESCRIPTION

Insect cells and lytic baculoviruses provide a proven method for high-level expression of full-length mammalian proteins. *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used to infect cultured insect cells (e.g. *Spodoptera frugiperda*). Expression of the highly abundant polyhedrin gene is non-essential in tissue culture and its strong promoter can be used for the transcription of foreign genes. The polyhedrin promoter is maximally expressed very late stage in infection when the lytic virus kills the host cells, resulting in high levels of expression even for certain toxic proteins. In addition, many post-translational modifications similar to those in mammalian cells are made in insect cells and proteins unable to be expressed in *E. Coli* have been successfully expressed in the insect cell system.

Orbigen's Sapphire™ Baculovirus DNA offers the following advantages:

- **Enhanced protein folding.** The disulfide isomerases gene was inserted at the p10 locus of the virus to ensure proper protein disulfide bond formation.
- **High recombinant efficiency.** The Sapphire™ Baculovirus DNA contains a lethal deletion of ORF1629 which can only result in viable viral particles if rescued by homologous recombination with a polyhedrin promoter-based transfer vector. This design significantly reduces time and effort in plaque assays.
- **High level expression.** The p10 promoter is partially deactivated. The lytic p10 gene is deleted so that transcription levels are higher due to reduced interference and healthier insect cells.

## PROTOCOLS: Co-transfection Using Insect Transfection Kit

1. Seed  $1 \times 10^6$  Sf9 (Cat#: CEL-10001) or Sf21 (Cat#: CEL-10002) cells onto each 35 mm tissue culture plate with 2 ml of Insect Cell Medium. Allow cells to attach firmly which take approximately 5 to 10 minutes.

**Note:** Prepare three plates for each co-transfection: one for the positive control, one for the negative control, and one for the recombinant plasmid of interest.

**Note:** Gently tilt plates in a side-to-side and back-and forth pattern to seed cells evenly. Do not swirl plates to avoid cell clustering.

2. Prepare DNA lipoplex transfection mixture: Add the following components in the order listed.

Serum Free Insect Cell Medium	1 ml
Insect DNA shuttle transfection reagent	5 ul
Sapphire™ Baculovirus DNA (500 ng)	5 ul
Transfer vector containing gene of interest	1 ug

Mix gently and let it sit at RT for 20 mins to allow DNA lipoplexes to form.

3. Co-transfection:

Just before the end of the transfection mixture incubation period, remove culture from 35 mm plate(s). Take care not to disturb cell monolayer by removing liquid from a tilted dish (For cells in serum-supplemented medium, wash the monolayer 2X 1ml serum-free medium right before co-transfection).

4. Remove medium from cells and immediately add 1 ml DNA lipoplex transfection mixture to each dish. Store 5~18 hrs in a 27°C humidified incubator and add 1 ml of TNM-FH medium to each plate.
5. Harvest medium containing recombinant baculovirus after 5 days. The expected titer of this initial P0 stock is about  $1 \times 10^6$  to  $1 \times 10^7$

## Amplification of Recombinant Baculovirus

The recombinant baculovirus need to be amplified to obtain a higher titer stock solution after co-transfection. Freshly seeded cells should be infected at a multiplicity of infection (MOI) of <1.

1. Infect  $5 \times 10^6$  cells per 10 cm plate (approximately 60% confluent) with 500 ul of transfection supernatant in 15 ml of TNM-FH medium supplemented with 10% FBS.
2. Incubate cells at 27°C for 3 days before harvesting.  
  
At 24 hrs post infection virus-infected cells are visibly swollen with enlarged nucleus which can be observed by light microscope. The medium will contain at least 107 virus particles per ml.
3. Perform the second run of amplification as described in step 1. Two rounds of amplification usually give a virus titer of  $2 \times 10^8$  Virus particles per ml of medium.



**Storage of Recombinant Baculoviruses:**

Use the following procedure for long-term storage of virus stocks:

1. Centrifuge viral stock at 4,000 x g to remove cellular debris.
2. If medium is serum-free, add serum to 10%.
3. Store viral stocks at 4°C. Virus titer, however, may decrease 5-10 fold during 6 months storage at 4°C.
4. Protect viral stocks from light to ensure maintenance of titer.
5. For long-term storage (up to 2 years), store small aliquots of virus at -80°C. Avoid repeated freezing and thawing.

**Titration of Amplified Virus Stocks:**

You must obtain a titer for a virus stock so that you can optimize subsequent infections to produce maximal yield of recombinant protein. Follow the end-point dilution assay outlined below to determine the titer of your virus stock.

1. Seed 2x10<sup>5</sup> Sf9 cells per well on a 12 well plate. Allow cells to attach firmly. Replace medium with fresh TMN-FH Medium (Cat#: MED-10001) containing 10% FBS.
2. Using the supernatant you wish to titer (usually obtained 5 days after the start of transfection), add 100 ul, 10 ul, 1 ul, and 0 ul to different wells of the plate.
3. Incubate the cells at 27°C for three days. Examine the cells for signs of infection (i.e. enlarged nucleus and swollen cells).
4. A successful transfection should give you uniformly large infected cells in all of the wells except the 0 ul wells (control). If you transfected with Sapphire™ DNA alone, all the wells should look like the 0 ul control well.
5. If only the 100 ul and 10 ul well seems to have infected cells and the 1 ul well looks more like the control, than the titer of your virus solution is low. Amplify the virus one more time before you proceed with protein production.
6. The cells from the 100 ul well can be harvested and lysed in Insect Lysis Buffer (Cat#: BUF-10014). The desired protein production may be checked on Western Blot (if antibodies are available) or by SDS-PAGE gel.

**Recombinant Protein Expression:**

Before producing the target protein on a large scale, characterize gene expression from the recombinant virus, and determine the time course of protein production. The table below provides a

guideline on various sizes of cell monolayers that can be used.

Type of Vessel	Cell Density*	Final Volume**
35 mm dish	0.7 x 10 <sup>5</sup>	1 ml
60 mm dish	2.5 x 10 <sup>6</sup>	3 ml
150 mm dish	2.0 x 10 <sup>7</sup>	30 ml
25 cm <sup>2</sup> flask	3.0 x 10 <sup>6</sup>	3 ml
75 cm <sup>2</sup> flask	5.0 x 10 <sup>6</sup>	10 ml
150 cm <sup>2</sup> flask	1.8 x 10 <sup>7</sup>	20 ml

\* Cell density in adherent culture is approximately 50% confluent.

\*\* Final volume includes culture medium and added virus. The amount of virus to add will depend on MOI.

We strongly recommended the use of insect cell line T. ni (Cat# CEL-10005) for protein expression. Always include an infection with wild-type virus and a mock infection as control. A high MOI (multiplicity of infection) is used to ensure synchronous infection and MOIs of 5, 10, and 20 should be tested. Most proteins expressed from the polyhedrin promoter reach their maximum levels somewhere between 48 hrs and 96 hrs post-infection; the best time to harvest depends on the nature of the target protein.

**RELATED PRODUCTS**

Product	Cat. No.
Sapphire™ Baculovirus DNA (5ug)	BVD-10001
Opal™ Baculovirus DNA (5ug)	BVD-10021
Opal™ Baculovirus DNA and Transfection Kit	BVD-10022
Sf9 Cells (in culture, 1x10 <sup>7</sup> cells)	CEL-10002
Sf9 Cells (frozen, 1x10 <sup>7</sup> cells)	CEL-10006
Sf21 Cells (in culture, 1x10 <sup>7</sup> cells)	CEL-10003
Sf21 Cells (frozen, 1x10 <sup>7</sup> cells)	CEL-10007
T. ni Cells (in culture, 1x10 <sup>7</sup> cells)	CEL-10005
T. ni Cells (frozen, 1x10 <sup>7</sup> cells)	CEL-10008
TNM-FH Insect Culture Medium, 1 L	MED-10001
Serum free Insect Culture Medium, 1 L	MED-10002
Serum free, Met free Insect Culture Medium, 1 L	MED-10003
pVL1392-Xyle Baculovirus Control Vector	BVP-10001
Insect Lysis Buffer 5X25 ml	BUF-10014

Please contact us for a complete list of life science research products and services.



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