Expression Systems

Transfection Medium
INSTRUCTIONS FOR USE

Product Description

Expression Systems' Transfection Medium is designed to complement ESF 921 and ESF AF insect cell culture media for the co-transfection, when using linearized baculovirus backbone systems, or transfection, when using bacmid systems, steps of baculovirus vector production. Transfection Medium is a serum-free, protein-free and animal product-free formulation designed to enhance DNA uptake by insect cells. Transfection Medium is appropriate for use with PEI and liposome based transfection reagents.

Product	Catalog Number	Volume	Storage	Shelf Life
Transfection Medium (1X), liquid	95-020-020 95-020-100	20 ml 100 ml	2°C to 8°C, protected from light	12 months from Date of Manufacture

Important Information

This protocol is optimized for Sf9 cells, but other Insect cell lines may be used successfully. All cell cultures used for transfection should be greater than 95% viability and be in the mid-logarithmic growth phase at the start of the procedure.

All steps of this procedure should be performed using aseptic technique in a biosafety cabinet.

Antibiotics inhibit transfection and should not be used during the incubation step with the transfection mixture. Antibiotics may be used once the transfection mixture is replaced with fresh medium.

Expression Systems Transfection Medium is formulated to maximize transfection efficiency of insect cells for generation of high titer P0 supernatants.

Safety Information

Read the Safety Data Sheets (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing and gloves.

Materials Required But Not Provided

Biosafety cabinet
Centrifuge
Micropipettes
Sterile polystyrene tubes
Tissue culture treated 6 well plate
27°C static incubator
Sterile centrifuge tubes
Transfer vector containing the gene of interest

Co-transfection Protocol

1. **Plate Cells:** Plate $0.9x10^6$ Sf9 cells in a well of a 6 well plate for each co-transfection and control reaction. Incubate the plate undisturbed at room temperature for 30 minutes to allow the cells to adhere. Co-transfection mixtures may be prepared during this incubation
2. **Prepare Co-transfection Mixtures:** For each co-transfection, pipette 100 µl **Transfection Medium** into each of two sterile polystyrene tubes, A and B. To tube A, add 5 µl (0.5 µg) **BestBac** DNA and 2 µg plasmid DNA. To Tube B, add 6 µl **Expres^{2}TR** transfection reagent. Incubate solutions A and B at room temperature for 5 minutes before combining the two solutions in one tube. Mix gently by slowly pipetting up and down two times using a large bore 1000 µl pipette tip. Incubate this mixture at room temperature for 20 minutes.
3. **Addition of Transfection Mixtures:** Add 800 µl **Transfection Medium** to each transfection mixture to increase the volume to 1000 µl. Aspirate the cell culture medium from each well and add the transfection solution to the appropriate well. To prevent drying of the monolayer, only aspirate and add transfection mixture to one well at a time.
4. **Incubation:** Incubate the plate at 27°C for 4-5 hours in a plastic bag or other sealed container with a damp paper towel or reservoir of water to maintain a humid environment
5. **Replace with Fresh Medium:** Aspirate the transfection solution and immediately replace with fresh cell culture medium. Antibiotics may be added at this point if desired. Incubate the plate at 27°C for 4-5 days in a plastic bag or other sealed container with a damp paper towel or reservoir of water to maintain a humid environment
6. **Harvest P0 virus:** Transfer supernatant to sterile centrifuge tubes, centrifuge supernatant at 2000 g for 5 minutes, and transfer to a sterile tube. Store the P0 virus at 4°C and determine the viral titer for further use. P0 titers generated using homologous recombination between linearized baculovirus backbone and complementary transfer vectors tend to range between 10^5-$5x10^7$ infectious units/ml.

Transfection Protocol

1. **Plate Cells:** Plate $0.9x10^6$ Sf9 cells in a well of a 6 well plate for each transfection and control reaction. Incubate the plate undisturbed at room temperature for 30 minutes to allow the cells to adhere. Transfection mixtures may be prepared during this incubation
2. **Prepare Transfection Mixtures:** For each transfection, pipette 100 µl **Transfection Medium** into each of two sterile polystyrene tubes, A and B. To tube A, add 1 µg bacmid DNA. To Tube B, add 6 µl **Expres^{2}TR** transfection reagent. Incubate solutions A and B at room temperature for 5 minutes before combining the two solutions in one tube. Mix gently by slowly pipetting up

and down two times using a large bore 1000 µl pipette tip. Incubate this mixture at room temperature for 20 minutes.

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6. **Harvest P0 virus:** Transfer supernatant to sterile centrifuge tubes, centrifuge supernatant at 2000 g for 5 minutes, and transfer to a sterile tube. Store the P0 virus at 4°C and determine the viral titer for further use. P0 titers generated using bacmid vectors tend to range between 10^6-5x10^8 infectious units/ml.

P0 Virus Operations

Subsequent steps taken with the P0 virus depend on the intended use. Below are typical actions taken with the P0 virus.

- Cells or supernatant from the P0 co-transfection may be screened for expression of recombinant protein, however expression levels might be low due to the sub-optimal expression conditions of the co-transfection.
- The P0 supernatant may be amplified to a larger volume high titer stock. See the protocol below.

Amplification To High Titer P1 Virus Stock

1. Seed a culture of Sf9 cells at a density of 1x10^6 cells/ml and incubate overnight at 27°C.
2. The following day, infect culture with P0 virus at a Multiplicity of Infection (MOI) of 0.1.
3. Incubate the culture for approximately 72 hours at 27°C.
4. Harvest P1 virus: pellet cells by centrifugation at 2000 g, pour off supernatant, and sterile filter through 0.2µm filter.
5. Store the P1 virus at 4°C and determine the viral titer for further use. For long term storage (years), aliquots of virus may be stored frozen at -80°C. Freezing and thawing of baculovirus supernatants will result in a drop in virus titer of approximately one log, but the titer will remain stable apart from this drop in titer.

Related Products

Product	Catalog Number
ESF 921	96-001
BestBac Linearized DNA	91-001 or 91-002
Expres^{2}TR Transfection Reagent	95-055
Baculovirus Titering Kit	97-101
Adapted Sf9 Cells	94-001 or 94-006

Important Licensing Information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Legend of Labeling Symbols

Symbol	Interpretation
	Catalog Number
	Lot Number
	*Research Use Only*
	Manufacturer
	Temperature Limitation
	Date of Manufacture
	Instruction for Use

Limited Product Warranty

Expression Systems LLC warrants that this product meets its specifications, as stated in our product brochures and certificates. This warranty lasts from the time we deliver the consumable until the completion of the consumables shelf life, when the product has been handled and stored in accordance with this IFU.

For technical assistance or documentation, such as Certificates of Analysis or Safety Data Sheets, email support@expressionsystems.com

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