Expression Systems
An Advancion Company

gp64-PE Antibody
INSTRUCTIONS FOR USE

Product Description

PE-labeled antibody specific for the baculovirus gp64 envelope protein is a useful tool for monitoring the infection status of the baculovirus-insect cell expression system. The AcMNPV baculovirus gp64 fusion protein is expressed on the surface of infected insect cells within six hours of infection. Assessing the percentage of infected insect cells 24 hours after infecting with a baculovirus allows for confirmation of the targeted MOI. When using a low MOI strategy, staining over a period of days allows for verification of viral spread.

Product	Catalog Number	Volume	Storage	Shelf Life
gp64-PE Antibody	97-201	gp64-PE Antibody, 100 µl	2°C to 8°C, protected from light	2 year recommended use by date

Important Information

This protocol is designed for use with a flow cytometer equipped to handle 96 well plates. If 96 well plate handling is not available, the protocol may be adapted for manual sampling. Staining may also be performed in 1.5 mL Eppendorf tubes or 4 mL snap cap tubes.

To verify the percentage of cells infected after a single round of infection, stain between 18-20 hours post infection.

This is a general protocol intended for experienced users of flow cytometry. Appropriate controls should be used to ensure the proper setting of gates for analysis.

Materials Required But Not Provided

Biosafety cabinet
96 well round bottom plate
Centrifuge with plate holders
Micropipettes, multi-channel pipette
Vacuum and aspirating pipettes
Flow cytometer equipped for Phycoerythrin detection
PBS-BSA (PBS supplemented with 1% Bovine Serum Albumin)
PBS

Staining Protocol

1. **Transfer Samples** to a 96 well round bottom plate. The number of cells per well should be approximately 2-3 x 10^5. Plate two wells per sample to be stained. One well will be used for an unstained control. Also plate two wells of uninfected cells.

2. **Dilute Antibody:** dilute gp64-PE antibody 1:200 in PBS-BSA. 50 µl diluted antibody will be required for one well of each sample. The replicate wells will receive 50 µl PBS-BSA.

3. **Stain Cells:** Centrifuge the plate at 500g for 5 minutes to pellet cells. Aspirate the supernatant taking care to not aspirate cells. Pipette 50 µl diluted antibody into wells to be stained. Pipette 50 µl PBS-BSA into control wells. Mix cells and solution gently by pipetting up and down, taking care to not aspirate air and create bubbles. Incubate the cells with the antibody for 20 minutes at 4°C.

4. **Wash:** pipette 200 µl PBS into wells containing cells and solution, centrifuge for 5 minutes at 500g, aspirate. Repeat PBS wash two more times. After aspirating PBS from the final wash, re-suspend cells in 200 ul PBS. Mix gently by pipetting up and down taking care to not aspirate air and create bubbles.

5. **Flow Cytometric Analysis:** Analyze the samples using a flow cytometer equipped for Phycoerythrin detection. Set the gates on the unstained control wells (% positive for PE should be less than 2).

Tips For Success

- All mixing steps should be done very gently with large bore pipette tips. Infected insect cells are more sensitive to shear stress than un-infected cells, so cell lysis caused by vigorous pipetting will artificially lower the percentage of PE positive cells and result in underestimation of infected cells.

- For best results, nearly all of the liquid should be aspirated from the wells during the wash steps.

- Make an effort to minimize the amount of time that elapses between aspiration of liquid from wells and addition of liquid. Allowing the cells to dry will negatively impact results

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Related Products

Product	Catalog Number
ESF 921	96-001
Adapted Sf9 Cells	94-001 or 94-006
BestBac™ Linearized DNA	91-001 or 91-002
Transfection Medium	95-020
Baculovirus Titering Kit	97-101
Titering Service	94-901

Legend of Labeling Symbols

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

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

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