

Grace's Insect Medium (2X)

Description

Grace's Insect Medium (2X) is a complete serum-free medium developed for the convenient and reproducible formulation of 1% agarose overlays used for plaque assays of baculovirus in a variety of Lepidopteran cell lines. When mixed as directed with agarose, such as 4% Agarose Gel, this medium concentrate allows convenient formulation of an overlay containing Grace's Insect Medium of proper osmolarity and 1X nutrient complement. Grace's Insect Medium (2X) is supplemented with L-glutamine, yeastolate, and lactalbumin hydrolysate.

Product	Catalog no.	Amount	Storage	Shelf life*
Grace's Insect Medium (2X), liquid	11667-037	100 mL	2°C to 8°C; Protect from light	12 months

* Shelf life duration is determined from Date of Manufacture.

Product use

For Research Use Only. Not for use in diagnostic procedures.

Safety information

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

Use

When mixed with 4% Agarose Gel according to directions, a 1X Grace's Insect Medium, 1% LMP (low melting point) agarose solution is produced. Either the nature or concentration of the agarose component may be easily varied by substituting a 4X concentrate of the desired agarose in water in the formulation of the overlay.

Prepare medium

- Supplementation of Grace's Insect Medium (2X) with L-glutamine is not necessary.
- Fetal Bovine Serum (FBS) is aseptically added to the medium to the desired concentration as part of preparing the agarose overlay.
- Antibiotics are not recommended; however 2.5–5 mL/L of Penicillin-Streptomycin may be used when required.

Prepare agarose overlay

The following plaque assay protocol is for preparing 50 mL (sufficient for 4 × 6-well plates, 2 mL per well) of a typical 1% agarose overlay using Grace's Insect Medium (2X), FBS, and 4% Agarose Gel.

Using strict aseptic technique:

1. Add 20 mL FBS to the 100-mL bottle of Grace's Insect Medium (2X).
2. Combine 30 mL of the Grace's Insect Medium (2X) with FBS, and 7.5 mL sterile culture grade water in a sterile 100-mL bottle. Cap securely and place in a 37°C water bath.
3. Melt 4% Agarose Gel in a 70°C water bath (approximately 10 minutes) and move to the 37°C water bath.
4. Working quickly to prevent the agarose from gelling, dispense 12.5 mL of the liquefied 4% Agarose Gel into the warm diluted Grace's Insect Medium with FBS, and mix by gentle swirling. Return to 37°C water bath.
5. After the appropriate viral inoculation incubation period, completely remove inoculum fluid from the insect cultures and gently replace with 37°C agarose overlay.
6. Allow overlay to harden (5–20 minutes), then place plates in a humidified environment and incubate at 28°C until plaques develop (4–7 days).










Note: The visual, immunologic, chemical, and microscopic characteristics of the plaque formation process and the methods for calculation of the inoculum titer and purification of individual plaques vary with the nature of the strain of baculovirus in use (see **References**).

Related products

Product	Catalog no.
4% Agarose Gel	18300
Sf9 Cells Adapted in Sf-900™ III SFM	12659
Distilled Water	15230
WFI for Cell Culture, USP	A12873
Gibco® Bottle, 100 mL	10339
FBS, Qualified , HI	16140
Sf21 Cells Adapted in Sf-900™ III SFM	12682
Penicillin-Streptomycin, liquid	15070
Sf-900™ III SFM, (1X)	12658
Sf-900™ II SFM, (1X)	10902
Sf-900™ Medium, 1.3X	10967
BaculoDirect™ N-Term Expression Kit	12562-054
BaculoDirect™ N-Term Transfection Kit	12562-062
BaculoDirect™ C-Term Expression Kit	12562-013
BaculoDirect™ C-Term Transfection Kit	12562-039
Bac-N-Blue™ Transfection Kit	K855-01
Bac-to-Bac® Baculovirus Expression System	10359
Bac-to-Bac® Vector Kit	10360

Explanation of symbols and warnings

The symbols present on the product label are explained below:

				
Temperature Limitation	Manufacturer	Batch code	Use By:	Catalog number
				
Caution, consult accompanying documents	Consult instructions for use	Keep away from light	Sterilized using aseptic processing techniques	

Limited product warranty

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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.

References

Luckow, Verne A. in *Recombinant DNA Technology and Applications* (ed. Ales Prokop, Rakesh K. Bajpai, Chester S.Ho; New York, McGraw Hill), 4:97-153, "Cloning and Expression of Heterologous Genes in Insect Cells with Baculovirus Vectors." ISBN: 0-07-029075-X (1991).

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support
For further assistance, email techsupport@lifetech.com

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