GIBCO™
Invitrogen Corporation

4% AGAROSE GEL
FOR CELL CULTURE OVERLAY APPLICATIONS

Cat. No.: 18300-012 **40 mL**

Storage Conditions: 2 to 8°C, in the dark.
Shelf life: 12 months

Background
The ability to produce high levels of functionally and immunologically competent product is a main factor in the growing popularity of the baculoviral expression vector system (BEVS). Since first described in 1983, hundreds of genes have been expressed by over 1000 researchers worldwide[1]. The successful expression of foreign genes in a baculovirus system requires exacting methods, reagents and materials. Included among these methods is the requirement for accurate and reproducible plaque titration and purification of recombinant virus. The identification and quantification of recombinant virus has been described as one of the most difficult aspects of the implementation of the BEVS system.
GIBCO BEVS Accessory Reagents are performance tested products which eliminate plaquing performance inconsistencies caused by raw material variability and formulation errors. These products improve the ability of the researcher to conveniently quantitate and purify recombinant baculovirus in either serum-free or traditional serum-supplemented systems.

Applications
Agarose has traditionally been dispensed as a powder to be measured, solubilized and autoclaved at the time of use. 4% Agarose Gel is a pre-measured, prepared and autoclaved gel form of LMP (low melting point) Agarose which needs only to be liquified at 70°C to be used in the building of plaquing overlays.
4% Agarose Gel melts at 65-70°C and remains fluid at 37°C. The product possesses many properties uniquely suited to the plaque assay and purification of baculovirus in insect cell lines. Because of the purity of the Agarose, cells proliferate and infect readily in its presence.
The low melting point of this Agarose allows liquid medium solutions containing it to be maintained in a liquid state without loss of labile components, as well as providing the ability to overlay the cells at moderate temperatures. The clarity of the gel state of this highly purified Agarose allows easy identification of OCC^{+} and OCC^{-} plaques as well as observation of single cell cytopathology.
When diluted with concentrated insect cell culture medium, 4% Agarose Gel produces a gel firm enough to immobilize local infections, yet ductile enough to allow for the efficient removal of plaques for clonal expansion.

Quality Control
Certificate of Analysis available upon request.

Instructions for Use
This plaquing protocol highlights (in steps A-C) the formulation of 60 mL of a typical Agarose overlay using 4% Agarose Gel. Users are referred to the many volumes, reviews and articles publicly available for more detailed and specific information pertinent to their particular application[1].

1. Prepare monolayers of insect cells on cell culture plates.
2. Inoculate plates with the appropriate dilutions of viral stock.
3. In an aseptic environment and using aseptic technique, prepare the Agarose overlay:

Materials:
- Insect Medium Concentrate (e.g. Sf900 Medium 1.3X, Cat. No. 10967, or Grace's Insect Medium 2X, Cat. No. 11667)
- Fetal Bovine Serum (Heat Inactivated), Cat. No. 16140 or 10438 (if required)
- 4% Agarose Gel, Cat. No. 18300
- Sterile cell culture grade water (if required)
- 37°C water bath
- 70 to 100°C water bath

4. Using strict aseptic technique:
   A. Dispense 45 mL of Sf-900 Medium, 1.3X** into a sterile 100 mL container and move to a 37°C water bath.
   B. Completely liquify 4% Agarose Gel in a 70°C water bath (approx. 10 min.), then move to the 37°C water bath.
   C. Dispense 15 mL of the liquified 4% Agarose Gel into the warm Sf-900 Medium, 1.3X and mix by gentle swirling. Return to 37°C water bath. Work quickly to prevent the Agarose from beginning to solidify.
5. After the appropriate incubation period, remove viral inoculum fluid from the insect cultures and gently replace with 37°C Agarose overlay.
6. Allow overlay to harden (5-20 min.), then place plates in a humidified environment and incubate at 28°C until plaques develop (4-5 days).

Note: The visual, immunologic, chemical and microscopic characteristics of the plaque formation process, methods for calculation of the inoculum titer, and purification of individual plaques vary with the nature of the strain of baculovirus in use. Refer to the many sources of detailed procedures available for this information. An excellent review is presented by V. Luckow[1].
**4% Agarose Gel may be melted and then diluted with warm cell culture quality water (Cat. No. 15230) to allow use with 2X formulations of growth medium such as Grace's Insect Medium, 2X. In this application, equal volumes of (1:1 diluted) LMP Agarose and 2X medium provides 1X LMP Agarose in 1X growth medium.
4% Agarose Gel may be re-solidified if necessary, and refrigerated for several weeks without loss of performance, if protected from light.

References:

[1] 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).

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You may also contact your Invitrogen Sales Representative or our World Wide Web site at www.invitrogen.com.

For research use only.
CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

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