3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

GENE THERAPY MEDIUM-3
For Adenovirus Production
Without L-glutamine

Product Code **G9916**
Storage Temperature 2-8 °C

Synonyms: Medium for Adenovirus Production

Product Description

Gene Therapy Medium-3 is a very low protein, serum-free, animal component-free medium for the production of adenovirus in cells of retinoblastomal origin. This medium will support high-density suspension cultures of retinoblastoma cells with minimal clumping. Additionally, the medium is designed to meet current regulatory guidelines for components used in the preparation of *in vivo* biotherapeutic agents.

Precautions and Disclaimer

For R&D use only. Not for drug, household or other uses.

MSDS is available upon request at: sigma-aldrich.com.
Pluronic is a registered trademark of BASF Corporation.

Components

Gene Therapy Medium-3 is devoid of animal-derived components. The proprietary formulation contains a small amount of recombinant insulin (20 ng/L) and polypeptides from plant sources. It also contains Pluronic F-68 (0.1%). This medium does not contain antibiotics.

Preparation Instructions

This medium is supplied as a sterile 1X liquid. Supplement the medium with 20 ml/L of 200 mM L-glutamine (Product Code G7513). Supplementation with a surfactant is not required.

Storage/Stability

The medium is stable, when stored at 2-8 °C and protected from light, until the date indicated on the label.

Procedure

Freezing and Thawing

HEK-293 and Y79 cell lines grown in Gene Therapy Medium-3 have been successfully frozen in liquid nitrogen and recovered. Cells must be in the mid-logarithmic phase of growth with greater than 90% viability.

1. Pellet cells by centrifugation for 5 minutes at 200 x g. Re-suspend the cells at a concentration of 3 x 10^6 to 5 x 10^6 cells/ml in 50% fresh Gene Therapy Medium-3 and 50% conditioned Gene Therapy Medium-3. Supplement the medium with DMSO at a final concentration of 7.5 - 10%.
2. Freeze cells in liquid nitrogen according to standard procedures (1 °C decrease per minute).
3. To recover cells, rapidly thaw the vial in a 37 °C water bath.
4. Dilute cells 1:10 in fresh Gene Therapy Medium-3. Mix by inversion.
5. Centrifuge the suspension at 200 x g for 5 minutes.
6. Aspirate supernatant and re-suspend the pellet in 1 ml of Gene Therapy Medium-3. Add 9 ml of fresh Gene Therapy Medium-3.
7. Transfer the cell suspension to a T-75 flask containing fresh Gene Therapy Medium-3.

Adaptation to Gene Therapy Medium-3

Adaptation of cells from serum-containing medium to serum-free (and protein-free medium) may be rapidly done with Gene Therapy Medium-3. It is critical that cell viability be at least 90% and that the cells are in the mid-logarithmic phase of growth during the weaning period.

1. Aspirate serum-containing medium from the cells. Detach cells by gently tapping the flask and gently triturate the cell suspension with a small-bore pipette to eliminate clumps. Determine cell viability and cell density with a hemacytometer and 0.4% trypan blue (Product No. T 8154).
2. To initiate cultures in Gene Therapy Medium-3, inoculate viable cells at a high density of 1 x 10^6 cells/ml. Incubate cultures at 37 °C in a humidified atmosphere of 5% CO_2. When cell density reaches 1.5 x 10^6 cells/ml, subculture three times a week.

3. For maintaining cultures in Gene Therapy Medium-3, seed stock cultures at 2 x 10^5 cells/ml for three days or at 3 x 10^5 cells/ml for two days.

NOTE: This maintenance schedule is appropriate for both attached and suspended cultures, including stirred-suspension systems.

Revision date: 2003-10