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-2
For HEK–293 cells
Without L-glutamine

Product Code **G0791**
Storage Temperature 2-8 °C

Synonyms: HEK Medium; 293 Medium; Medium for Adenovirus Production in HEK-293 cells

Product Description

Gene Therapy Medium-2 is a very low protein, serum-free, animal component-free medium for the production of adenovirus in HEK-293 cells. The medium is designed to support high-density suspension cultures of HEK-293 cells with minimal clumping. Additionally, it meets current regulatory guidelines for components used in the preparation of *in vivo* biotherapeutics agents.

Intended Use

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

Components

Gene Therapy Medium-2 is devoid of animal-derived components. The proprietary formulation contains a small amount of recombinant insulin (2 mg/L) and polypeptides from plant sources. It also contains Pluronic® F-68 (0.1%). This medium does not contain antibiotics. The addition of antibiotics is recommended when using the medium with primary cells.

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 cells grown successfully in Gene Therapy Medium-2 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-2 and 50% conditioned Gene Therapy Medium-2. 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-2. Mix by inversion.
5. Centrifuge the suspension at 200 x g for 5 minutes.
6. Remove supernatant and re-suspend the pellet in 1 ml of Gene Therapy Medium-2. Add 9 ml of fresh Gene Therapy Medium-2.
7. Transfer the cell suspension to a T-75 flask containing fresh Gene Therapy Medium-2 at a final volume of 30 ml.

Adaptation to Gene Therapy Medium-2

Adaptation of cells from serum-containing medium to serum-free (and protein-free medium) may be rapidly done with Gene Therapy Medium-2. 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 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 Code T8154).

2. To initiate cultures in Gene Therapy Medium-2, inoculate viable cells at a high density of 1 x 10^6 cells/ml.
3. Incubate cultures at 37 °C in a humidified atmosphere of 5% CO_2.
4. When cell density reaches 1.5 x 10^6 cells/ml, subculture three times a week.
5. For maintaining cultures in Gene Therapy Medium-2, 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 suspension cultures, including stirred-suspension systems.

Precautions and Disclaimer

MSDS is available upon request at: **sigma-aldrich.com**.

Pluronic is a registered trademark of BASF Corporation.

04/02