

PRODUCT DESCRIPTION

Human Embryonic Kidney (HEK)293 cells are a commonly used mammalian cell line for the expression of recombinant proteins and antibodies. Serum-Free HEK Cell Culture Media has been optimized for the growth and expansion of HEK293 cells under serum-free, low protein (<75 µg/mL) conditions in suspension culture. This is a complete media that will support growth of a wide variety of HEK293 cells without further supplementation. Other cytokines and growth factors may be supplemented to facilitate specific cell applications.

Note: Cytokines and growth factors can be obtained from R&D Systems® (www.rndsystems.com/proteins).

INTENDED USE

Serum-free HEK Cell Culture Media is formulated to support recombinant protein and antibody expression through expansion of HEK293 cells in suspension culture under serum-free conditions. This media has been tested for its ability to support high-density suspension cultures of HEK293EBNA cells adapted from adherent culture platforms.

STABILITY & STORAGE

Upon receipt, this media should be stored at 2-8 °C. Protect from light. Use within the expiration date listed on the bottle. For optimal culture performance, bottles should be used within 1 month after opening to prevent loss of CO₂ and elevated pH.

ENDOTOXIN LEVEL AND OSMOLARITY

Endotoxin level of < 1.0 EU/mL.

Osmolarity range of 280-320 mOsm/kg.

PRECAUTION

This product contains human transferrin. This transferrin was tested at the donor level using an FDA licensed method and found to be non-reactive for anti-HIV-1/2 and Hepatitis B surface antigen. As no testing can offer complete assurance of freedom from infectious agents, these reagents should be handled as if capable of transmitting infection. When handling biohazardous materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- This reagent should not be used beyond the expiration date indicated on the label.

PROCEDURE FOR THE EXPANSION OF HEK293 CELLS

The protocol below describes the expansion of HEK293 cells in Serum-free HEK Cell Culture Media.

Note: *This protocol must be read in its entirety before using this product.*

Serum-free HEK293 Cell Culture Media is not designed for transfection use.

For transfection it is recommended to use Serum-free HEK293 Transfection Media, Catalog # CCM024.

OTHER MATERIALS REQUIRED

- HEK293 cells, such as HEK293EBNA
- Penicillin-Streptomycin (100X), optional
- Non-baffled shake flasks
- 15 mL centrifuge tubes
- Serological pipettes
- 37 °C and 5% CO₂ humidified incubator
- Centrifuge (low speed clinical or equivalent)
- Hemocytometer
- Rotary shaker, circular orbit, 1" orbital diameter
- Water bath

REAGENT PREPARATION

Serum-free HEK Cell Culture Media - Serum-free HEK Cell Culture Media is complete and ready for use. If desired, add Penicillin-Streptomycin at a 1:100 dilution. **Note:** *If Penicillin-Streptomycin is not needed for the experiment, it can be omitted.*

PROCEDURE

Note: *This procedure describes the expansion of HEK293 cells in suspension culture.*

1. Warm Serum-free HEK Cell Culture Media to 37 °C.
2. Determine the size and number of flasks needed for plating. Cells should be inoculated at 0.5×10^6 cells/mL. For example, 12.5×10^6 cells in 25 mL of media in a 125 mL non-baffled shaker flask.
3. Incubate the shaker flask on the rotary shaker at 110-120 RPM at 37 °C/5% CO₂.
4. Count cells daily to monitor cell density and viability.
5. When cell density reaches $1.5\text{-}2.5 \times 10^6$ cells/mL, expand the culture to a 1 L non-baffled shake flask containing Serum-free HEK Cell Culture Media to inoculate a final cell density of $\sim 0.5 \times 10^6$ cells/mL.
6. Repeat steps 3-4.
7. When cell density reaches $1.5\text{-}2.5 \times 10^6$ cells/mL, expand the culture to a 2 L non-baffled shake flask containing Serum-free HEK Cell Culture Media to inoculate a final cell density of $\sim 0.5 \times 10^6$ cells/mL.
8. Culture can continue as described in steps 6-7 to expand into higher volumes as desired.
9. When the final desired volume is reached, continue to culture cells 3-6 days depending on application need. Without supplementation, final cell density at maturation is expected to reach $\sim 4.0 \pm 0.5 \times 10^6$ viable cells/mL by day 5-6.

DATA EXAMPLES

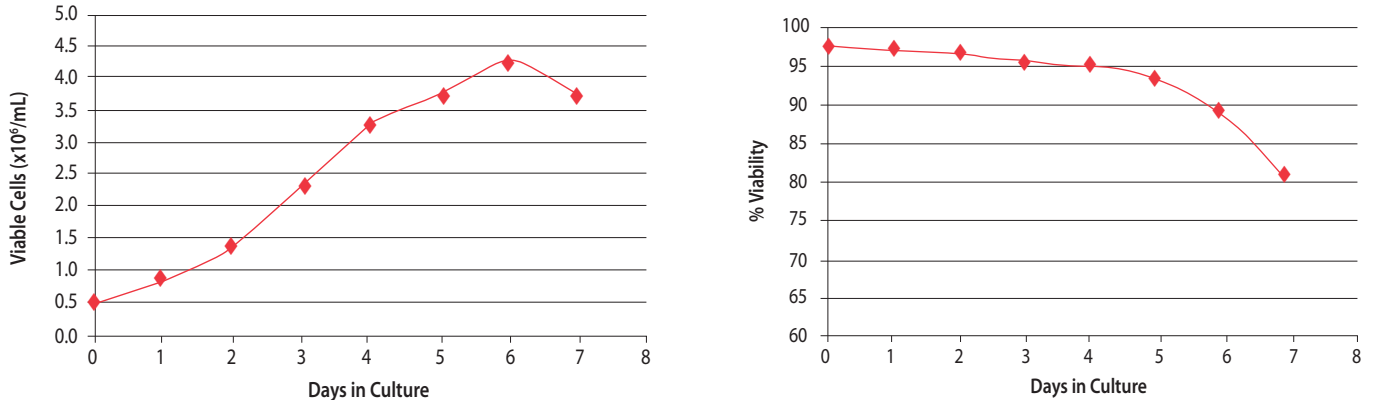


Figure 1: Serum-free HEK Cell Culture Media Supports Cell Growth of HEK293EBNA Cells. 25x10⁶ HEK293EBNA cells were inoculated in 50 mL of Serum-free HEK293 Cell Culture Media. Cells were cultured at 37 °C/5% CO₂ for 7 days. The cell growth curves show the viable cell count/mL (A) and % viability (B) at each day of culture.

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