

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 Transfection Media has been optimized for the transfection of HEK293 cells under serum-free, low protein (<75 µg/mL) conditions in suspension culture. This is a complete media that will support transfection 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 Transfection Media is formulated to support transfection of HEK293 cells for recombinant protein and antibody expression in suspension culture under serum-free conditions. The media has been tested for its ability to support transfection of 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.

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 TRANSFECTION OF HEK293 CELLS

The protocol below describes the transfection of HEK293 cells in Serum-free HEK Transfection Media.

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

Serum-free HEK293 Transfection Media contains components optimized for cell transfection.

For expansion, it is recommended to use Serum-free HEK293 Cell Culture Media, Catalog # CCM023.

OTHER MATERIALS REQUIRED

- HEK293 cells, such as HEK293EBNA
- Serum-free HEK293 Cell Culture Media (R&D System, Catalog # CCM023)
- 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 Transfection Media - Serum-free HEK Transfection 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 transfection 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 125mL 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 $1.5 - 2.5 \times 10^6$ cells/mL, the culture can be expanded with a seeding cell density of 0.5×10^6 cells/mL into a higher volume, or cells can be used for transfection.
6. To transfect, cells are spun down at 1500 rpm for 5 minutes.
7. Resuspend the cells at $3 - 5 \times 10^6$ cells/mL density in the appropriate volume of **Serum-free HEK Transfection Media**.
8. Perform transfection according to desired transfection reagent/system.
9. Incubate the transfected cells on a rotary shaker at 110-120 RPM at 37°C/5% CO₂ for a period of time per specific transfection protocol (e.g. ~3 hours).
10. After the transfection time, add an equal volume of **Serum-free HEK Cell Culture Media** to allow the cells to recover for additional screening (for stable transfection) or feed the cells to allow the culture to continue for protein or antibody expression.

DATA EXAMPLES

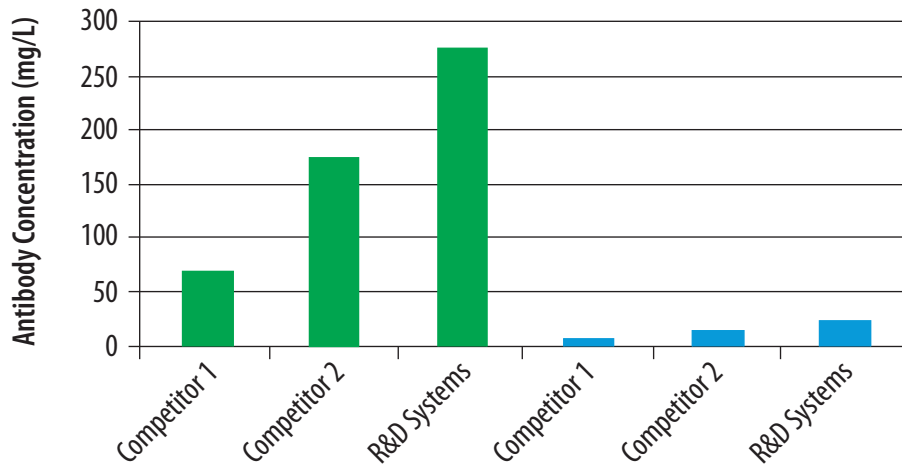


Figure 1: Serum-free HEK Transfection Media Supports Higher Antibody Expression in HEK293 Cells. HEK293 cells were expanded to a concentration of $2-3 \times 10^6$ cell/mL in Serum-free HEK Cell Culture Media (R&D Systems®, Catalog # CCM023). Cells were then spun down and resuspended at 5×10^6 cells/mL in Serum-free HEK Transfection Media or one of two other commercially available medias (Competitor 1 and Competitor 2). Each cell suspension was then transfected with a plasmid encoding one of two different antibodies (Antibody1-green bars; Antibody2-blue bars). After transfection, each culture was diluted with an equal volume of cell culture media, either competitor's or Serum-free HEK Cell Culture Media (R&D Systems®, Catalog # CCM023). The cultures were maintained for an additional 5-7 days prior to assessing antibody expression by ELISA.

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