Tribio HEK293 Suspension Transient Medium (TBS8110)

Catalog	Unit Size
TBS8110-1L	1L
TBS8110-10L	10L
TBS8110-20L	20L

Description

Tribio HEK293 Suspension Transient Medium is a chemically defined, animal-component-free, serum-free, and hydrolysate-free medium for suspension culture of HEK293 and other mammalian cells. It is optimized for transient transfection and viral vector production, including AAV and lentivirus, and works well with polycationic transfection reagents (We recommend our TBS8042 TransReagent Maxi) such as PEI. The medium can be used for protein expression, from cell thawing and expansion to transfection and production using a single medium across scales.

Application

- Suspension culture of HEK293 and other mammalian cell lines.
- Transient transfection applications.
- Viral vector production, including AAV and lentivirus.
- Gene therapy research and manufacturing workflows.
- Protein and antibody expressions.

Components and Specification

With L-Glutamine, with growth factor, chemically defined, free of animal-derived components, free of hydrolysates and proteins, free of antibiotics.

Procedure

1. Recommend Culture Conditions

The recommended culture conditions are provided in Table 1.

Table 1: Recommended culture conditions.

Parameter	Condition
Shaker diameter	5 cm
Shaker speed	125 – 185 rpm
Temperature	37 °C
CO_2	5%

Based on the setup described in Table 1, the working volumes for different sizes of polycarbonate Erlenmeyer shake flasks were determined (Table 2). For cell lines prone to strong aggregation, baffled shake flasks can be used; however, a reduction in shaking speed may be required for this configuration.

2. Routine Culture Maintenance and Cell Expansion

a. Place an adequate volume of culture medium into a polycarbonate Erlenmeyer shake flasks and allow it to equilibrate for 1 hour (refer to Tables 1 and 2 for applicable parameters).

b. Measure the viable cell concentration of the seed culture and calculate the volume required for inoculation. The desired starting density is 2–5 × 10^5 viable cells/mL

Table 2: Recommended culture working volumes in various shake flask sizes.

Size of shaker	Shape	Working Volume
125 mL	plain, vent cap	20 – 50 mL
250 mL	plain, vent cap	80 – 150 mL
500 mL	plain, vent cap	200 – 300 mL
1000 mL	plain, vent cap	400 – 600 mL

c. Adjust the volume of medium in the shake flask by removing the calculated amount so that the final culture volume matches the target working volume after inoculation. Refer to Table 2 for working volumes corresponding to shaker size.

d. Initiate the culture by inoculating to a final concentration of 2–5 × 10^5 cells/mL.

e. Maintain the culture under the incubation conditions specified in Table 1.

f. Subculture the cells when the viable cell density reaches 15–40 × 10^5 cells/mL, typically every 3–4 days.

g. If cell growth is limited or cell density remains low during the adaptation period, collect the cells by centrifugation after 3–4 days and replace the medium without further dilution.

Note: Medium equilibration may not be necessary for all cell lines. In some cases, direct use of chilled culture medium stored at 2–8 °C has been shown to support improved performance and can minimize variability associated with the equilibration step.

3. Bioreactor Cultivation

To achieve optimal growth, the inoculation density in a bioreactor for batch culture should be maintained between 3–6 × 10^5 cells/mL. For HEK293 cell cultures, recommended starting conditions are pH 7.0–7.5, dissolved oxygen (DO) at 30–40%, and a temperature of 37 °C.

4. Cell Cryopreservation and Recovery

a. Cryopreservation

- Select healthy cultures with viability >90%.
- Prepare freezing medium: 90% HEK293 Suspension Transient Medium + 10% DMSO, pre-cooled to 2–8 °C.
- Harvest cells (115 × g, 5 min), discard supernatant.
- Resuspend cells to 1 × 10^7 cells/mL and aliquot 1.5 mL per cryovial.

- Place vials at –80 °C for 24 hours, then transfer to long-term storage (–140 to –196 °C).

b. Recovery

- Rapidly thaw cryovials in a 37 °C water bath.
- Transfer contents to a centrifuge tube containing 20 mL HEK TF medium.
- Centrifuge at 115 × g for 5 min, discard supernatant.
- Resuspend cells in 10 mL fresh medium.
- Adjust cell density to 5–10 × 10^5 cells/mL and inoculate into an appropriate shake flask or vented tube for continued culture.

5. Medium and Cell Adaptation

a. Direct Adaptation from Serum-containing Adherent Cultures

1. Expand cells in standard serum-containing medium.
2. Harvest enough cells to inoculate a suspension culture at 4–6 × 10^5 cells/mL by centrifuging at 200 × g for 5 minutes and discard the supernatant.
3. Resuspend the cells in serum-free medium.
4. Subculture or replace the medium every 2–4 days, depending on cell density and viability.
5. Continue passaging until cell viability stabilizes above 90% and growth rates remain consistent over 3–5 passages.
6. For optimal performance, inoculate adapted cells at 2–5 × 10^5 cells/mL. Dilute cultures every 3–4 days. Because HEK cells tend to aggregate, cultures should be maintained under agitation using spinner bottles, shaker flasks, or similar systems.

b. Gradual Adaptation from Serum-containing Adherent Cultures

1. Expand cells in standard serum-containing medium.
2. Collect sufficient cells to inoculate a suspension culture at 4–6 × 10^5 cells/mL by centrifuging at 115 × g for 5 minutes and remove the supernatant.
3. Resuspend cells in HEK TF medium containing 2% fetal bovine serum (FBS).
4. Subculture or refresh the medium every 2–4 days, depending on cell density.
5. After at least 3 passages, reduce the serum concentration to 0.5%.
6. Continue subculturing or changing medium every 2–4 days based on cell density.
7. After 2–4 additional passages, decrease the serum concentration to 0%.
8. Maintain the culture until viability remains above 90% and growth rates are stable for 3–5 passages.
9. Adapted cells should be seeded at 2–5 × 10^5 cells/mL for optimal growth. Dilute cultures every 3–4 days. Due to HEK cell aggregation, agitation is recommended using spinner bottles, shaker flasks, or comparable cultivation systems.

c. Direct Adaptation from Suspension Cultures in Chemically Defined Medium

1. Expand the cells in a chemically defined standard medium.
2. Collect enough cells to inoculate a new suspension culture at 4–6 × 10^5 cells/mL by centrifuging at 115 × g for 5 minutes, then discard the supernatant.
3. Resuspend the cell pellet in fresh chemically defined medium.
4. Refresh the culture by subculturing or replacing the medium every 2–4 days, based on cell density.
5. Continue the process until cell viability remains above 90% and growth rates are consistent over 3–5 passages.
6. For best performance, inoculate adapted cells at a density of 2–5 × 10^5 cells/mL. Cultures should be diluted every 3–4 days. Because newly adapted HEK cells tend to form aggregates, agitation is recommended using spinner bottles, shaker flasks, or equivalent cultivation systems.

Storage

Store with the lid tightly closed, protected from light, at 2–8 °C. Do not freeze.

Relative Products

TBS8024 MCF10 Cell Complete Medium

TBS8027 EMEM with L-Glutamine

TBS8030 McCoy's 5A Medium With L-Glutamine, and Phenol Red

TBS8031 30% BSA Solution in DPBS

TBS8036 Inoue Transformation Buffer for Preparation of Competent Cells

TBS8047 MCF-7 Cell Complete Medium

TBS8057 SOB Medium

TBS8058 SOC Medium

TBS8061 DMEM-high Glucose Medium

TBS8063 RPMI-1640 Medium

TBS8083 DMEM.F12, HEPES

This product is for research use only.