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CREATE THE LIFE SCIENCE KYOKUTO PHARMACEUTICAL INDUSTRIAL CO., LTD.

KP293 type 2 Expression Medium for HEK293 Cells
【Transient Expression Culture Protocol】

【Precautions for Use and Handling】

(1) Perform all operations under sterile conditions. Avoid freezing this product and store it according to the storage instructions (2–10 °C, protected from light.).
(2) Do not use the reagents if they have been frozen, as this may cause deterioration and lead to unexpected performance.
(3) Do not use reagents past their expiration date.
(4) Do not use the product if the container is damaged or if any foreign matter is found inside. When disposing of this product, treat it as infectious waste.
(5) Disposal methods vary by location, so please follow the regulations of your local authority.
(6) It is recommended that the cell line be adapted to this medium prior to use.
(7) The seeding density described in this protocol is an example based on KYOKUTO's culture conditions. Optimal conditions may vary depending on the characteristics of the cell line and the specific culture environment; therefore, preliminary optimization is recommended.

【Culture Conditions】

Table 1. Culture Conditions

Culture temperature	37 ℃±0.5℃
CO_2 concentration	5 -8%CO_2
Culture area	125 mL flask
Medium volume	20-30 mL
Shaking speed	125-135 rpm

【Thawing Procedure】

This procedure describes the steps for thawing cells that have been adapted to KP293 type 2 Expression Medium (hereafter referred to as KP293 type 2).

1. Aliquot the required volumes of KP293 type 2 into centrifuge tubes in advance and store them at room temperature or in an incubator prior to use.
2. Rapidly thaw the frozen 293 cells in a 37 °C water bath.
3. Transfer the thawed cells into the centrifuge tube containing KP293 type 2 for cell resuspension.
4. Centrifuge at 150 × g for 5 minutes and discard the supernatant.
5. Resuspend the cell pellet in an appropriate volume of KP293 type 2 for culture.
6. Determine the cell number and viability.
7. Proceed with subculture. (Table 2).

【Subculture】

When the cell density reaches 3.0 × 10^6 cells/mL or higher, perform subculture.
(Recommended cell density: 3.0–5.0 × 10^6 cells/mL)

Table 2. Standard seeding cell number for routine passaging

Days after subculture	Recommended Seeding Density (cells/mL)
Day 3	0.4-0.5 × 10^6
Day 4	0.3-0.4 × 10^6

1. Aliquot the required volume (30 mL) of KP293 type 2 into a centrifuge tube in advance, and store at room temperature or in an incubator prior to use.
2. Count the number of 293 cells in culture and calculate the seeding density shown in Table 2.
3. Seed the cells into a new flask so that the total volume of fresh medium is 30 mL at the calculated seeding density.
4. After 3–4 days of culture, repeat the subculture.

【Adaptation Culture】

Adaptation from other serum-free media to KP293 type 2 can be achieved by repeating the following procedure for passages 3–4.
A temporary decrease in cell growth (around passages 2–3) may occur; however, growth will recover with continued subculture.

※ It is more reliable to gradually increase the proportion of KP293 type 2 to 50%, 70%, 80%, and then 90%.

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Table 3. Seeding density for adaptation

Days after subculture	Recommended Seeding Density (cells/mL)
Day 3	0.5-0.6 × 10^6
Day 4	0.4-0.5 × 10^6

1. Aliquot the required volume (30 mL) of KP293 type 2 into a centrifuge tube in advance, and store at room temperature or in an incubator until use.
2. Count the number of 293 cells in culture and calculate the seeding density shown in Table 3.
3. Seed the cells into a new flask so that the total volume of fresh medium is 30 mL at the calculated seeding density.
4. After at least 3–4 passages, continue with routine subculture.

【Cryopreservation】

1. Count the number of 293 cells in culture and calculate the required volume of freezing medium (KP293 type 2 + 10% DMSO) based on the viable cell density.
   ※ Please use cells that are in the logarithmic growth phase for cryopreservation.
   ※ Ensure that cell viability is 90% or higher.
2. Prepare the freezing medium and keep it at 4°C until use.
3. Centrifuge the 293 cells (150 × g, 5 min) and discard the supernatant.
4. Resuspend the cells in freezing medium to a final concentration of 1.0 × 10^7 cells/mL.
5. Dispense 1 mL of the cell suspension into each cryovial.
6. Freeze the vials at a cooling rate of approximately –1°C/min using an appropriate freezing container, then transfer them to –80°C.
7. Store the cells for 24–48 hours after freezing. After that, be sure to transfer them to the vapor phase of liquid nitrogen.

【Transfection】

Please follow the protocol provided for each transfection reagent.

1. Adjust the cell number according to the recommended seeding density for the transfection method.
2. Confirm the viable cell density and viability prior to transfection (viability ≥ 95% recommended).
3. Collect the supernatant at the appropriate time after transfection.

KYOKUTO PHARMACEUTICAL INDUSTRIAL CO., LTD.
7-8, Nihonbashi Kobuna-cho, Chuo-ku, Tokyo, 103-0024, JAPAN
https://www.kyokutoseiyaku.co.jp/

COSMO BIO USA.
2792 Loker Avenue West, Suite 101, Carlsbad, CA 92010 USA
Phone: +1 760-431-4600 / Fax: +1 760-431-4604
Email: support@cosmobiousa.com
URL: https://www.cosmobiousa.com/