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Protein Expression Medium (PEM)

Description

Protein Expression Medium (PEM) is specifically formulated for the large-scale high-density suspension culture of PER.C6® human embryonic retinoblast cells and the production of recombinant proteins. PEM is an animal origin-free, serum-free medium formulated without components directly derived from human or animal sources (PEM contains one low-level recombinant protein).

Product	Catalog No.	Amount	Storage	Shelf Life*
Protein Expression Medium (PEM)	12661-013	1000 mL	2°C to 8°C; Protect from light	12 months

* Shelf Life duration is determined from Date of Manufacture.

Product Use

For Research or Further Manufacturing Use. Not for use in diagnostic procedures.

Important Information

PER.C6® cells are immortalized human embryonic retinoblast cells carrying the E1 minigene of adenovirus type 5.

Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Prepare Medium

PEM requires supplementation with 4mM GlutaMAX™-I or L-glutamine.

1. Aseptically add 2 mL of 200 mM GlutaMAX™-I or L-glutamine to 100 mL of PEM before use.
2. Add antibiotics, if required.

Addition of a surfactant such as Pluronic® F-68 is not required.

Once supplemented, store the complete PEM at 2°C to 8°C protected from light.

Culture Conditions

Media: Protein Expression Medium (PEM).

Cell Line(s): PER.C6® Cells, HEK 293 Cells, and HeLa Cells.

Culture Type: Suspension.

Culture Vessels: Shake flask, roller bottle or bioreactor.

Temperature Range: 36°C to 38°C.

Incubator Atmosphere: Humidified atmosphere of 8% CO_2 in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Recovery

1. Rapidly thaw (<1 minute) frozen vial of cells in a 37°C water bath.
2. Transfer the entire contents of the cryovial into a 125-mL shake flask containing 24.5 mL of prewarmed PEM supplemented with 4 mM GlutaMAX™-I or L-glutamine.
3. Incubate at 37°C in a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 125–135 rpm. Loosen flask caps to allow for gas exchange.
4. Subculture cells in mid-logarithmic phase 3–5 days post-thaw at a seeding density of 3×10^5 viable cells/mL. Subculture cells a minimum of 3 passages before use in other applications.

Note: Do not centrifuge PER.C6® cells upon thawing as they are extremely fragile during recovery from cryopreservation.

Subculture Suspension Cultures

1. Determine viable cell density using a Countess® Automated Cell Counter.
2. Seed cells at $2–3 \times 10^5$ viable cells/mL in sterile culture vessels containing prewarmed complete PEM (30 mL per 125-mL shake flask).
3. Incubate at 37°C in a humidified atmosphere of 8% CO_2 on an orbital shaker platform rotating at 115–135 rpm. Loosen flask cap to allow for gas exchange.
4. Passage cells every 3–5 days into fresh prewarmed complete PEM.

Note: PER.C6® cells may exhibit some minor clumping (~10 cells per clump), vortex samples vigorously for up to 45 seconds before counting to obtain accurate cell proliferation data. Optimal vortexing conditions must be individually determined by the user to maximize cellular viability. Do not vortex cells to be subcultured.

Note: It is recommended to thaw a fresh low-passage vial of cells every 3 months or 30 passages.

Adapt PER.C6® Cells to PEM Suspension Culture

Most PER.C6® cell lines will adapt directly from conventional serum supplemented or other serum-free medium. It is critical that cell viability be ≥90% and the growth rate be in mid-logarithmic phase prior to initiating adaptation procedures. PER.C6® cells grown in PEM typically demonstrate a population doubling time of less than 40 hours and achieve cell densities in excess of 2×10^6 cells/mL in shaker culture and $3–4 \times 10^6$ cells/mL in roller bottle or bioreactor culture. Individual results may vary.

Direct Adaptation of PER.C6® Cells to PEM

1. Subculture PER.C6® cells grown in conventional medium with 5–10% serum or other serum-free medium into PEM. During the adaptation procedure use a seeding density of $3–4 \times 10^5$ viable cells/mL.
2. Incubate at 37°C in a humidified atmosphere of 8% CO_2 on an orbital shaker platform rotating at 115–135 rpm. Loosen caps of flasks to allow for gas exchange.
3. Subculture cells as necessary 4–6 days post seeding once cells have demonstrated growth.
4. Continue to passage cells at $3–4 \times 10^5$ viable cells/mL until consistent growth is achieved.
5. After several passages in 100% PEM, the viable cell count should reach $2–3 \times 10^6$ cells/mL with a viability exceeding 85% within 4–6 days of culture. At this stage the culture is considered to be adapted to PEM. The seeding density may be reduced to $2–3 \times 10^5$ viable cells/mL during the final stages of adaptation.

Note: If suboptimal performance is achieved using the direct adaptation method, use the sequential adaptation (weaning) method.

Sequential Adaptation of PER.C6® Cells to PEM

1. Subculture PER.C6® cells grown in conventional medium with 5–10% serum or other serum-free medium into a 25:75 ratio of PEM to the original media. During the adaptation procedure use a seeding density of 3–4 × 10^5 viable cells/mL.
2. Incubate at 37°C in a humidified atmosphere of 8% CO_2 on an orbital shaker platform rotating at 115–135 rpm. Loosen caps of flasks to allow for gas exchange.
3. Subculture 4–6 days post seeding. Once consistent cell growth has been achieved, passage cells into a 50:50 ratio of PEM to original medium.
4. Repeat step 3 of this procedure, stepwise increasing the ratio of PEM to original medium (75:25 followed by 90:10) until the cells are transferred into 100% PEM. Multiple passages at each step may be needed.
5. After several passages in 100% Protein Expression Medium, the viable cell count should reach 2–3 × 10^6 cells/mL with a viability exceeding 85% within 4–6 days of culture. At this stage the culture is considered to be adapted to PEM. The seeding density may be reduced to 2–3 × 10^5 viable cells/mL during the final stages of adaptation.

Cryopreservation

1. Prepare the desired quantity of cells in a tissue culture flask, harvesting in mid-log phase of growth with viability >90%. Reserve the conditioned medium to prepare cryopreservation medium.
2. Use Countess® Automated Cell Counter to determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of 0.5 × 10^7 cells/mL.
3. Prepare the required volume of cryopreservation medium of 92.5% PEM (50:50 ratio of fresh to conditioned media) +7.5% DMSO on day of intended use, and store at 4°C until use.
4. Harvest cells by centrifugation at 100 × *g* for 5–10 minutes. Resuspend the pellet in the pre-determined volume of 4°C cryopreservation medium.
5. Dispense aliquots of this suspension into cryovials according to the manufacturer's specifications (i.e., 1.5 mL in a 2-mL cryovial).
6. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. Transfer frozen cells to liquid nitrogen (vapor phase); storage at –200°C to –125°C is recommended.

Note: Check viability of cryopreserved cells 24 hours after storage of vials in liquid nitrogen (See **Recovery**).

Related Products

Product	Catalog No.
293-F Cells, SFM Adapted	11625
T-REx™-HeLa Cell Line	R714-07
GlutaMAX™-I, 200 mM (100X), Liquid	35050
L-Glutamine-200 mM (100X), Liquid	25030
Adenovirus Expression Medium	12582
Pluronic® F-68, 10% (100X)	24040
Anti-Clumping Agent	0010057
Countess® Automated Cell Counter	C10227
Trypan Blue Stain	15250

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

				
Protect from light	Manufacturer	Batch code	Use By:	Catalog number
				
Caution, consult accompanying documents	Consult instructions for use	Temperature Limitation	Sterilized using aseptic processing techniques	

Limited Use Label License: Internal Research and Bioproduction Use

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product (a) to perform internal research for the sole benefit of the purchaser; and (b) to culture cells for the purpose of producing a product wherein the product will be used for any or all of the following: (i) internal research use by the purchaser; (ii) resale for internal research use by third parties; (iii) performance of research conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties; (iv) resale for use as a human therapeutic agent or diagnostics product or component by third parties; (v) performance of manufacturing services conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties.

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