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life technologies

Adenovirus Expression Medium (AEM)

Description

Adenovirus Expression Medium (AEM) is an animal-origin free media specifically formulated for the high-density suspension culture of PER.C6® human embryonic retinoblast cells and the high-titer production of replication-incompetent adenovirus and influenza virus. AEM also supports the use of PER.C6®, HEK 293, and HeLa cells in the production of recombinant proteins and monoclonal antibodies. AEM is a serum-free medium formulated without components directly derived from human or animal sources enabling users to maximize experimental results with less labor-intensive suspension cultures while eliminating many of the concerns associated with the use of serum or other primary, animal-origin, materials.

Product	Catalog no.	Amount	Storage	Shelf life*
Adenovirus Expression Medium (AEM)	12582-011	1000 mL	2°C to 8°C; Protect from light	18 months

* Shelf Life duration is determined from Date of Manufacture.

Product use

Caution: For manufacturing, processing, or repacking.

Important information

- PER.C6® cells are immortalized human embryonic retinoblast cells carrying the E1 minigene of adenovirus type 5.
- AEM contains one low-level recombinant protein.

Safety information

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

Prepare medium

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

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

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

Culture conditions

Media: Adenovirus Expression Medium (AEM).

Cell line(s): PER.C6® Cells, 293 Cells.

Culture type: Agitated suspension or bioreactor.

Culture vessels: Shake flasks, spinner bottles, 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.

Adapt PER.C6® cells to serum-free suspension culture

PER.C6® cells grown in AEM 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 3×10^6 –4×10^6 cells/mL in roller bottle or bioreactor culture. Individual results may vary. PER.C6® cells may exhibit some minor clumping (~10 cells/clump), vortex samples vigorously for up to 45 seconds before passaging or counting to obtain accurate cell proliferation data. Optimal vortexing conditions must be individually determined by the user to maximize cellular viability.

1. Aspirate medium from PER.C6® monolayer culture and discard.
2. Add 5–10 mL prewarmed (37°C) supplemented AEM to culture flask. Gently wash cell monolayer.
3. Remove AEM and add sufficient prewarmed TrypLE™ Select without phenol red to cover the monolayer.
4. Incubate 2 minutes, remove the TrypLE™ Select and re-incubate flask at 37°C for up to 10 minutes or until cells have fully detached.
5. Observe cell monolayer using an inverted microscope to ensure complete cell detachment from the surface of the flask.
6. Resuspend cells in 10 mL prewarmed supplemented AEM, transfer to a sterile 15-mL centrifuge tube, and centrifuge the cell suspension at 100 × *g* for 5 minutes.
7. Discard supernatant and resuspend the cell pellet in fresh, prewarmed, supplemented AEM. Determine the viable cell density using a Countess® Automated Cell Counter. (Alternative automated or manual methods may be used.)
8. If aggregation is apparent upon microscopic examination, triturate with a small bore pipette or vortex until (most) cell clumps are dispersed into a single-cell suspension.
9. Dilute to the desired final cell density and volume in prewarmed supplemented AEM.

Shaker culture

1. Seed cells at 3×10^5 viable cells/mL in sterile 125-mL Erlenmeyer shake flasks to a maximum total volume of 20 mL of pre-warmed supplemented AEM per flask.
2. Incubate the shake flask(s) on an orbital rotary shaker at 75–85 rpm in a humidified atmosphere of 8% CO_2 in air at 37°C.
3. Monitor cell density and dilute to 2×10^5–3×10^5 cells/mL with prewarmed supplemented AEM whenever the cell density approaches 1.5×10^6 cells/mL (about every 3–4 days).
4. It is recommended to subculture the cells a minimum of two passages before use.

   Note: After adaptation to growth in serum-free suspension culture, it is possible to scale up the cultures in spinner flasks and/or bioreactors.

Viral vector production

AEM has demonstrated that it has the capacity to support adenovirus production. Using a Multiplicity of Infection (MOI) of 5 and a cell concentration of 1×10^6 cells/mL, typical yields of infectious particles ranged between 10^6–10^9 particles/mL for 2–5 days after infection.

Related products

Product	Catalog No.
GlutaMAX™-I (100X), liquid	35050
L-Glutamine-200mM (100X), liquid	25030
Protein Expression Medium (PEM)	12661
293-F Cells, SFM Adapted	11625
293fectin™ Transfection Reagent	12347
TrypLE™ Select (1X), no Phenol Red	12563
Anti-Clumping Agent	0010057
Trypan Blue Stain	15250
Countess® Automated Cell Counter	C10227

Explanation of symbols and warnings

The symbols present on the product label are explained below:

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

Limited Product Warranty

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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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