gibco
by life technologies

CD 293 (1X)

Description

CD 293 medium is a chemically-defined, animal origin-free, protein-free medium optimized for the growth of high-density suspension cultures of HEK 293 cells. CD 293 Medium contains no proteins or peptides of animal, plant, or synthetic origin; or hydrolysates or components of undefined composition simplifying downstream purification of virus and recombinant proteins. CD 293 contains a proprietary dispersant to minimize cell clumping. CD 293 Medium is formulated without phenol red to minimize potential estrogen-like effects of phenol red.

Product	Catalog no.	Amount	Storage	Shelf Life*
CD 293	11913-019	1000 mL	2°C to 8°C; Protect from light	12 months
CD 293 AGT™	12529-020	1 L	2°C to 8°C; Dark and Dry	24 months
	12529-012	1 × 10 L	2°C to 8°C; Dark and Dry	24 months
	12529-001	1 × 100 L	2°C to 8°C; Dark and Dry	24 months
	12529-003	10 kg	2°C to 8°C; Dark and Dry	24 months

*** Shelf Life duration is determined from Date of Manufacture.**

Product Use

Caution: For manufacturing, processing, or repacking.

Important Information

CD 293 medium is not recommended for adherent culture.

Safety Information

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

Prepare Media

Reconstitute CD 293 AGT™:

1. Add the entire contents of a 1 L package of CD 293 AGT™ to 900 mL room temperature deionized or distilled water. Mix for 30 minutes or until completely dissolved.
2. Add deionized or distilled water to final volume of 1000 mL.
3. Sterilize by 0.2 µm pore size membrane filtration.
4. Store at 2°C to 8°C. Protect from light.
5. Aseptically supplement with L-glutamine or GlutaMAX™-I at time of use (see **Supplement Media**).

Note: CD 293 AGT™ contains sodium bicarbonate. *Do not* add additional sodium bicarbonate. CD 293 AGT™ is auto pH and osmolality adjusted, no further adjustment required. For final lot pH and osmolality specifications please refer to Certificate of Analysis specification.

Supplement Media

- CD 293 medium requires aseptic supplementation with L-glutamine or GlutaMAX™-I to a final concentration of 4 mM (20 mL/L of a 200 mM stock solution) before use.
- Supplementation with antibiotics is not recommended as they may reduce growth rate.
- The addition of a surfactant such as Pluronic® F-68 is not required.

Culture Conditions

Media: CD 293 medium, supplemented

Cell Line: HEK 293 Cells

Culture Type: suspension

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, cells may have a reduced growth rate at lower CO_2 (i.e., 5% CO_2) levels. Ensure proper gas exchange and minimize exposure of cultures to light.

Recovery

1. Rapidly thaw (<1 minute) frozen vial of cells (7.5×10^6 cells) in a 37°C water bath.
2. Transfer the entire contents of the cryovial into a sterile disposable 125-mL Erlenmeyer shake flask containing 18 mL prewarmed complete CD 293 medium.
3. Incubate at 37°C in a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 125 rpm. Loosen flask caps to allow for gas exchange.
4. Subculture cells 3–5 days post thaw.

Subculture

1. Determine viable cell density using a Countess® Automated Cell Counter. Alternate methods (e.g. Coulter counter or hemocytometer) may also be used.
2. When viable cell density reaches ~1.5×10^6 cells/mL, dilute cells to 2–3 × 10^5 cells/mL with pre-warmed complete CD 293. Dispense cell suspension, up to a maximum of 20 mL/flask, into sterile 125-mL Erlenmeyer shake flasks.
3. Incubate the shake flask(s) on a rotary shaker (125–130 rpm) at 37°C in a humidified atmosphere of 8% CO_2 in air.
   Note: Cells may have a reduced growth rate at lower CO_2 (i.e., 5% CO_2) levels.

Adapt HEK 293 Cells from Adherent-Dependent Culture to Suspension Culture

Note: Life Technologies offers 293-F and 293-H cells which have been pre-adapted to growth in CD 293 medium.

Adapt HEK 293 Cells from Adherent-Dependent Culture to Suspension Culture, continued

1. Aspirate media from cell monolayer and displace HEK 293 cells from the flask's surface by rapping the flask sharply against your hand or a protected surface several times. **Note:** Do not use trypsin or other proteolytic agents to dislodge cells.
2. Resuspend dislodged cells in 5 mL of CD 293. **Note:** HEK 293 cells cultured in CD 293 may grow as 2–10 cell clusters.
3. Disperse clusters into a single-cell suspension by triturating with a small bore pipette or vortexing before passaging or counting. Optimal vortexing conditions must be determined based upon speed and duration versus viability.
4. Determine viable cell density using a Countess® Automated Cell Counter. Alternate methods (e.g. Coulter counter or hemocytometer) may also be used.
5. Dilute cells in pre-warmed complete CD 293 medium to a viable cell density of 1×10^6 cells/mL.
6. Incubate the shake flask(s) on a rotary shaker (125–130 rpm) at 37°C in a humidified atmosphere of 8% CO_2 in air.
7. Monitor viable cell density daily. When the viable cell density reaches ~1.5×10^6 cells/mL, dilute to 2.5–3.0 × 10^5 cells/mL with pre-warmed medium. Continue to dilute to 2.5–3.0 × 10^5 cells/mL whenever the viable cell density reaches ~1×10^6 cells/mL. After several passages of consistent growth and viability in CD 293 the culture is considered to be adapted.

After adaptation to growth in serum-free suspension culture, it is possible to scale-up the cultures in spinner flasks or bioreactors. The appropriate spinner or impeller speed should be individually determined.

Caution: Some spinner apparatus emit significant heat and water-jacketed incubators usually cannot readily equilibrate to temperature variations. Temperatures ≥40°C are lethal to HEK 293 cells.

Cryopreservation

1. Prepare the desired quantity of cells, harvesting in mid-log phase of growth with viability >90%. Reserve the conditioned medium to prepare cryopreservation medium.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of 0.5–1 × 10^7 cells/mL.
3. Prepare the required volume of cryopreservation medium of 92.5% CD 293 medium (50:50 ratio of fresh to conditioned media) + 7.5% DMSO and store at 4°C until use. **Important:** Prepare cryopreservation medium on the day of intended 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. Immediately dispense aliquots of this cell suspension into cryovials according to the manufacturer's specifications (i.e., 1 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).

Related Products

Product	Catalog no.
L–glutamine, 200mM (100X), liquid	25030
GlutaMAX™-I (100X), liquid	35050
293 F Cells, SFM Adapted	11625
293 H Cells, SFM Adapted	11631
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 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.

The purchase of this product does not grant the purchaser any additional rights, including (without limitation) the right to transfer or resell the product in any form, the right to use the product as a therapeutic agent or diagnostics test component, or to use the product to perform other tests on a contract or fee per test basis for or on behalf of third parties. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support
For further assistance, email **techsupport@lifetech.com**

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