CD Hybridoma Medium

Catalog Numbers 11279023, 12372025, 12372017, 12372001, 12372003

Pub. No. MAN0007307 Rev. 3.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

Gibco™ CD Hybridoma Medium is a chemically-defined, protein-free medium optimized for the growth of a variety of hybridomas and myelomas and the production of monoclonal antibodies in stationary or agitated suspension systems. CD Hybridoma Medium contains no proteins (animal, plant, or synthetic origin), hydrolysates, or components of unknown composition. CD Hybridoma Medium is formulated without phenol red to minimize the estrogen-like effects of phenol red.

Contents and storage

Product	Cat. No.	Amount	Storage	Shelf life[1]
CD Hybridoma Medium (1x)	11279023	1000 mL	2°C to 8°C; Protect from light	18 months
CD Hybridoma AGT™ Medium[2]	12372025	1 L	2°C to 8°C; Store dark and dry	24 months
	12372017	10 L		
	12372001	100 L		
	12372003	10 kg		

[1] Shelf Life duration is determined from Date of Manufacture.
[2] AGT= Advanced Granulation Technology

Culture conditions

Media: Complete CD Hybridoma Medium

Cell line: Hybridoma, Myeloma

Culture type: Suspension

Culture vessels: Shake flasks, spinner bottles or bioreactor

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 5–10% CO_2 in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Procedural guidelines

- Cholesterol dependent cell lines (e.g., NS0 and derivatives) require additional supplementation of CD Hybridoma Medium with a cholesterol supplement or some other source of cholesterol (i.e., Cholesterol Lipid Concentrate (250X)).
- CD Hybridoma Medium and CD Hybridoma AGT™ Medium are formulated without L-glutamine for use in Glutamine Synthetase Expression Systems.
- CD Hybridoma Medium contains an organic (anionic) iron carrier with a high absorption at 280 nm. Antibody detection and/or purification protocols should be designed to take this into account. We recommend pre-screening the medium to determine potential interference. Do not use ammonium sulfate precipitation as a means of purification.

Prepare medium

Reconstitute medium

1. Place a 2-L graduated cylinder containing a magnetic stir bar on magnetic stir plate.
2. Fill the graduated cylinder with 900 mL of distilled water.
3. Turn on magnetic stir plate to form a vortex.
   The vortex formed will be about 1/4 to 1/3 the height of the cylinder.
4. Add 19.2 g/L of CD Hybridoma AGT™ Medium to make 1 L and mix for 10–15 minutes until CD Hybridoma AGT™ Medium goes into solution.
5. Test and record the pH.

6. Bring the reconstituted medium to 1 L and mix for about 3–5 minutes.

7. Sterile filter through a 0.2 micron filter.

   Note: Use low protein binding, low extractable filter.

Supplement media

- Aseptically add L-glutamine or GlutaMAX™ I Supplement, 8 mM final concentration (40 mL/L), to the medium before use.

  Note: Consider using lower levels of L-glutamine if using a fed batch or perfusion protocol or if the cell line in use is sensitive to ammonia.

- The use of antibiotics is not recommended. Most general antibiotics are compatible with CD Hybridoma Medium including penicillin/streptomycin, gentamicin, anti-PPLO, linocin and Amphotericin B if necessary.

  IMPORTANT! Do not use kanamycin sulfates, neomycin sulfates or penicillin/streptomycin/neomycin mixtures.

- The addition of a surfactant such as Pluronic™ F-68 is not required.

- – Once supplemented, store the complete CD Hybridoma Medium at 2°C to 8°C protected from light.

Recovery

1. Rapidly thaw (<1 minute) frozen cells in a 37°C water bath.

2. Transfer the entire contents of the cryovial into a 125-mL shake flask containing 28.5 mL of pre-warmed complete CD Hybridoma Medium.

3. Incubate at 37°C in a humidified atmosphere of 5–10% CO_2 in air on an orbital shaker platform rotating at 125–135 rpm. Loosen flask caps (or use vented caps) to allow for gas exchange.

4. Subculture cells in mid-logarithmic growth phase 3–5 days post-thaw at a seeding density of 3×10^5–5×10^5 viable cells/mL. Subculture cells a minimum of 3 passages before use in other applications.

   Note: Do not centrifuge hybridoma cells during recovery as they are extremely fragile upon thawing.

Subculture suspension culture

1. Determine viable cell density using a Countess™ Automated Cell Counter (or alternative automated or manual methods).

2. Seed cells at 2×10^5–3×10^5 viable cells/mL in sterile culture vessels containing pre-warmed complete CD Hybridoma Medium (30 mL per 125-mL shake flask).

3. Incubate at 37°C in a humidified atmosphere of 5–10% CO_2 in air on an orbital shaker platform rotating at 125–135 rpm. Loosen flask cap to allow for gas exchange.

4. Subculture cells when viable cell density reaches $\geq 1 \times 10^6$ viable cells/mL into clean, sterile flask(s) with fresh pre-warmed complete CD Hybridoma Medium.

Adapt hybridoma cells to CD Hybridoma Medium

It is critical that cell viability be at least 90% and cells be in the mid-logarithmic growth phase prior to adaptation. Successful adaptation will depend upon the particular hybridoma cell line and the culture conditions employed. It is recommended that backup cultures in the original medium be maintained until success with the new medium has been achieved.

Direct adaption

1. Subculture hybridoma cells grown in conventional medium with 5–10% serum or other serum-free medium into pre-warmed complete CD Hybridoma Medium. During the adaptation procedure seeding density should be double the normal seeding density for the cell line.

2. Monitor cell growth until viable cell density reaches 1×10^6 viable cells/mL. Subculture the cells to a viable cell density of 3×10^5–6×10^5 viable cells/mL in fresh pre-warmed complete CD Hybridoma Medium.

3. Continue to monitor and passage cells for 3–5 passages until consistent growth is achieved.

   Note: If suboptimal performance is observed using the direct adaptation method over 3–5 passages, use the sequential adaptation method.

Sequential adaption

Follow the procedures (“Subculture suspension culture” on page 2) with the following modifications.

1. During the adaptation procedure use a seeding density of 4×10^5–5×10^5 viable cells/mL.

2. Subculture cells into stepwise increasing ratios of complete CD Hybridoma Medium to original medium with each subsequent passage (25:75, 50:50, 75:25, 90:10 followed by 100% CD Hybridoma Medium). Multiple passages at each step may be needed.

   After several passages in 100% CD Hybridoma Medium, the viable cell count should exceed 1×10^6–2×10^6 cells/mL with a viability exceeding 85% within 4–6 days of culture. At this stage the culture is considered to be adapted to CD Hybridoma Medium. The seeding density may be reduced to 2×10^5–3×10^5 viable cells/mL during the final stages of adaptation.

Cryopreservation

Prepare the desired quantity of cells harvesting in mid-log phase of growth with viability >90%. Reserve the conditioned medium to prepare cryopreservation medium.

1. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of 0.5×10^7–1×10^7 cells/mL.

2. Prepare the required volume of cryopreservation medium of 92.5% CD Hybridoma 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.

3. Harvest cells by centrifugation at 100 × *g* for 5–10 minutes. Resuspend the cell pellet in the pre-determined volume of 4°C cryopreservation medium.

4. Immediately dispense aliquots of this suspension into cryovials according to the manufacturer's specifications.

5. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).

6. Transfer frozen cells to liquid nitrogen, (vapor phase) storage at –200°C to –125°C is recommended.

Related product

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
L-Glutamine, 200 mM (100X)	25030081
GlutaMAX™ I Supplement (100X)	35050061
Cholesterol Lipid Concentrate (250X)	12531018
Penicillin-Streptomycin	15070063
Gentamicin	15750060
Antibiotic-Antimycotic (100X)	15240062
Amphotericin B	15290018
Pluronic™ F-68, 10% (100X)	24040032
Water, Distilled	15230001
Anti-Clumping Agent	0010057AE

Note: A Hybridoma Medium Master file has been submitted to the FDA. Permission to cross reference the Master file may be obtained by contacting Technical Support or your local Sales Representative.

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 at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0007307

Revision	Date	Description
3.0	23 March 2022	Updated Reconstitue CD Hybridoma AGT™ Medium workflow. Updated formatting. Removed LULL 517 statement. Updated Use statement.
2.0	14 July 2015	Updated SKU shelf life to 18 months, updated LULL, rebranded