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by Thermo Fisher Scientific

CD Hybridoma Medium

Description

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 estrogen-like effects of phenol red.

Product	Catalog no.	Amount	Storage	Shelf life*
CD Hybridoma Medium (1X), liquid	11279-023	1000 mL	2°C to 8°C; Protect from light	18 months
CD Hybridoma AGT™**	12372-025	1 L	2°C to 8°C; Store dark and dry	24 months
	12372-017	10 L	2°C to 8°C; Store dark and dry	24 months
	12372-001	100 L	2°C to 8°C; Store dark and dry	24 months
	12372-003	10 kg	2°C to 8°C; Store dark and dry	24 months

*Shelf Life duration is determined from Date of Manufacture. **AGT= Advanced Granulation Technology.

Product use

Caution: For manufacturing, processing, or repacking.

Important information

- 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., 250X Cholesterol Lipid Concentrate).
- 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.

Safety information

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

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.

Prepare medium

Reconstitute CD Hybridoma AGT™:

1. Add entire contents of a single 1 L package of CD Hybridoma AGT™ to 900 mL room temperature deionized or distilled water. Mix 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.
   Note: Use low protein binding, low extractables filter.

Note: CD Hybridoma AGT™ Medium contains sodium bicarbonate. **Do not add** additional sodium bicarbonate. CD Hybridoma AGT™ Medium 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

- Aseptically add L-glutamine or GlutaMAX™-I, 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 Fungizone™ antimycotic 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 cultures

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 adaptation

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 adaptation

Follow the procedures for *Subculture Suspension Cultures* 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, 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 products

Product	Catalog no.
L–Glutamine, 200mM (100X), liquid	25030
GlutaMAX™-I (100X), liquid	35050
250X Cholesterol Lipid Concentrate	12531
Penicillin-Streptomycin, liquid	15070
Gentamicin Reagent Solution (50 mg/mL), liquid	15750
Antibiotic-Antimycotic (100X), liquid	15240
Fungizone™, liquid	15290
Pluronic™ F-68, 10% (100X)	24040
Water, Distilled	15230
Anti-Clumping Agent	0010057
Countess™ Automated Cell Counter	C10227

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 you local Sales Representative.

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 No. 517: Internal and Bioproduction Use

Notice to Purchaser: The purchase of this product conveys to the purchaser the limited, non- transferable right to use the purchased amount of the product to (a) perform internal research for the sole benefit of the purchaser; (b) manufacture protein (or other biological material) for resale; and (c) perform research or manufacturing services conducted by the purchaser on a fee for service or contract basis for or on behalf of third parties. However, the purchaser may transfer this product, its components, or materials made using this product to a third party (including contract research/manufacturing organizations), provided that each such third party agrees in writing to use such product, components, or materials solely on behalf of the purchaser, and such third party is restricted from further transferring any such product, components, or materials to any individual or entity other than the purchaser. No additional rights are granted. By purchasing this product, the purchaser agrees not to: (1) resell the product in any form; (2) use the product as a therapeutic agent or diagnostics test component; (3) reverse engineer the product or cause the product to be reverse engineered; or (4) use the product for purposes other than what is indicated in this Limited Use Label License. The purchaser is responsible for obtaining all regulatory approvals necessary for any therapeutic or diagnostic use of the protein (or biological material) manufactured using this product. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

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