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Dynamis™ AGT™ Medium

Description

Dynamis™ AGT™ Medium is specifically designed to offer the highest batch and fed-batch culture performance and yield with recombinant CHO cells in a chemically defined environment. The medium is formulated without hypoxanthine and thymidine for use in dihydrofolate reductase (DHFR)-amplified systems, without L-glutamine for use in glutamine synthetase systems, and without phenol red to minimize estrogen-like effects of phenol red. Furthermore, the glucose concentration is formulated to minimize potential lactic acid accumulation under typical culture conditions. The chemically defined, protein-free, animal origin component-free Dynamis™ AGT™ Medium provides the power to achieve high titers, start process development faster, and streamline or simplify transfer to manufacturing scale.

Product	Catalog no.	Amount	Storage	Shelf life*
Dynamis™ AGT™ Medium	A26175-04 A26175-01 A26175-02 A26175-03	1 × 1 L 1 × 10 L 1 × 100 L 10 kg	2°C to 8°C; Store dark and dry	12 months

* Shelf Life duration is determined from Date of Manufacture.

Product use

Caution: For manufacturing, processing, or repacking.

Safety information

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

Culture conditions

Media: Dynamis™ AGT™ Medium

Cell line: CHO cells

Culture type: Suspension

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 8% CO_2 in air. Ensure that proper gas exchange is achieved in culture vessels and minimize exposure of cultures to light.

Culture vessels: Shake flasks, spinner bottles (rpm may vary with shaker platform/impeller design and should be empirically determined for optimal cell growth), or bioreactor.

Reconstitute Dynamis™ AGT™ Medium

1. Measure 90% of the final volume deionized or distilled water at room temperature (15°C to 30°C).
2. Add Dynamis™ AGT™ Medium at 24.8 grams/L to water.
3. Mix for a minimum of 30 minutes.
4. Using a calibrated vessel, dilute to final production volume with ambient deionized or distilled water. Mix for an additional 10 minutes.
5. Measure the pH and check and record osmolality.
6. Sterilize immediately by membrane filtration (positive pressure recommended).

   Note: Once the product is filtered, use immediately or store at 2 to 8°C for up to 6 months. Protect from light.

Prepare complete medium

Dynamis™ AGT™ Medium requires aseptic supplementation with L-glutamine or GlutaMAX™-I prior to use.

1. Add GlutaMAX™-I or L-glutamine at 2–8 mM final concentration to the medium before use.
2. Add 10 mL/L of HT Supplement for use in applications not requiring DHFR amplification.
3. Glucose supplementation may be required for terminal batch cultures and should be determined empirically.
4. Add Anti-Clumping Agent (1 mL/L) to the medium after transfection to reduce cell aggregation, if required.

   Note: Consider reducing L-glutamine concentration for fed-batch or perfusion protocols, or if the cell line in use is sensitive to ammonia. Addition of a surfactant such as Pluronic® F-68 is not required.

Recover frozen cells

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 30 mL pre-warmed complete Dynamis™ AGT™ Medium.
3. Incubate at 37°C in a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 115–135 rpm.
4. Maintain a cell density of 0.5×10^6–1×10^6 viable cells/mL for the first two passages following recovery; thereafter, return to your normal maintenance schedule.

   Note: Do not centrifuge the cells after thawing as they are extremely fragile upon recovery from cryopreservation.

Subculture cells

1. Determine viable cell density using a Countess® Automated Cell Counter (alternate automated or manual methods may also be used).
2. Ensure that the cell density is ≥1×10^6 viable cells/mL, viability is ≥90%, and the growth rate is in mid-logarithmic phase prior to subculturing.
3. Calculate the volume of cell culture and medium necessary to seed a flask at 2×10^5–3×10^5 viable cells/mL in a total volume of 30 mL fresh Dynamis™ AGT™ Medium per 125-mL shake flask.

   Note: If cell density does not reach 1×10^6 viable cells/mL within 5 days of recovery, centrifuge cells at 100 × *g* for 5 minutes and resuspend the cell pellet in 20–30 mL of fresh complete Dynamis™ AGT™ Medium.
4. Incubate at 37°C in a humidified atmosphere of 8% CO_2 in air on an orbital shaker platform rotating at 115–135 rpm.
5. For optimal performance and cell growth, dilute the cells at a seeding density of 3×10^5 viable cells/mL every 3–4 days with fresh Dynamis™ AGT™ Medium.

   Note: We recommend thawing a fresh, low-passage vial of cells for use every 3 months or 30 passages.

Adaptation of CHO cells to Dynamis™ AGT™ Medium

We recommend adapting CHO cells to Dynamis™ AGT™ Medium using sequential adaptation. However, some CHO cell lines will adapt directly from 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.

Direct adaptation

1. For direct adaptation of CHO cells grown in other serum-free medium into Dynamis™ AGT™ Medium, dilute cells into 100% Dynamis™ AGT™ Medium using a seeding density of 3×10^5–4×10^5 viable cells/mL when subculturing (see **Subculture cells**).
2. Continue to subculture cells at 3×10^5–4×10^5 viable cells/mL (every 3–4 days) until consistent growth is achieved. Once cell growth has been demonstrated, the seeding density may be reduced to 2×10^5–3×10^5 viable cells/mL during the final stages of adaptation.
3. After several passages in Dynamis™ AGT™ Medium, the viable cell count should reach at least 2×10^6 cells/mL with ≥85% viability within 3–4 days of seeding culture. At this stage, the culture is considered to be adapted to Dynamis™ AGT™ Medium.

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

Sequential adaptation

1. During sequential adaptation of CHO cells grown in conventional 5–10% serum-supplemented medium or other serum-free medium, use a seeding density of 3×10^5–4×10^5 viable cells/mL.
2. Monitor cell growth using Countess® Automated Cell Counter until viable cell density reaches ≥1×10^6 cells/mL.
3. Dilute cells using a 25:75 ratio of complete Dynamis™ AGT™ Medium to the original medium. We recommend maintaining backup cultures in the original ratio medium until success with the new ratio medium is achieved. At each subsequent passage, dilute cells with stepwise increasing ratios of complete Dynamis™ AGT™ Medium to original medium (25:75, 50:50, 75:25, 90:10, followed by 100% Dynamis™ AGT™ Medium). Multiple passages at each step may be needed to achieve consistent growth.
4. After several passages in 100% Dynamis™ AGT™ Medium, the viable cell count should reach at least 2×10^6 cells/mL with ≥85% viability within 3–4 days of seeding culture. At this stage, the culture is considered to be adapted to Dynamis™ AGT™ Medium.

Cryopreservation

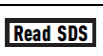
1. Prepare the desired quantity of cells, harvesting in mid-log phase of growth with >90% viability. Reserve the conditioned medium to prepare cryopreservation medium.
2. Determine the viable cell density and calculate the required volume of cryopreservation medium to give a final cell density of >1×10^7 cells/mL.
3. Prepare the required volume of cryopreservation medium of 92.5% Dynamis™ AGT™ Medium (50:50 ratio of fresh complete to conditioned media) + 7.5% DMSO and store at 4°C until use. **Important**: Prepare cryopreservation medium on the day of 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.
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.

   Note: Check viability of cryopreserved cells 24 hours after storage of vials in liquid nitrogen (see **Recover frozen cells**).

Related Products

Product	Cat. No.
L-glutamine, 200mM (100X), liquid	25030
GlutaMAX™-I, (100X), liquid	35050
HT Supplement, (100X), liquid	11067
Anti-Clumping Agent	0010057
Freedom® CHO-S® Kit	A13696-01
EfficientFeed™ C+ AGT™ Supplement	A25031

Explanation of Symbols and Warnings

				
Caution, consult accompanying documents	Temperature Limitation	Keep away from light	Use By:	Consult instructions for use
				
Batch Code	Catalog number	Manufacturer	Read Safety Data Sheet	

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Limited Use Label License No. 545: Chemically Defined Cell Culture Media

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For further assistance, email **techsupport@lifetech.com**.