SagiCHO™

Chemically Defined Fed-Batch Medium

SagiCHO™ is a chemically-defined medium designed for high density suspension culture of Chinese Hamster Ovary (CHO) cell lines. It is free of any animal-origin components, hydrolysates, proteins, growth factors and components of unknown composition. This medium supports the high level expression of recombinant proteins and therapeutic antibodies. When used in conjunction with OPM's next generation high performance feeds, SagiCHO® Feed or VegaCHO™ Feed, enhanced cell growth, improved viability and increased expression levels of target molecules can be achieved.

Application

SagiCHO™ cell culture medium supports the cultivation and maintenance of high density suspension culture, enabling high titer production in fed-batch processes. This medium is intended for large-scale manufacturing of therapeutic biomolecules and for research purposes. It is not intended for use in humans, diagnostic procedures, or therapeutic purposes.

Storage & Transportation

Store at 2-8°C in a dry environment and protected from light
Liquid media is shipped at room temperature and dry powder media on blue ice

Shelf Life

SagiCHO™ Medium Liquid: 12 months
SagiCHO™ Powder: 24 months

Reconstitution Protocol for SagiCHO™ Powder Medium

1. Fill a clean mixing vessel to to 90% of the final volume with high quality purified water at room temperature (25°C to 35°C), such as WFI at ambient temperature. For example, to prepare 1 liter of SagiCHO™, start with 900 mL of water. Start mixing.
2. Add SagiCHO™ DPM at 21.73 g/L slowly to the vessel, avoiding formation of clumps. Mix for 10 minutes. Ensure that residue powder on the vessel wall is incorporated into the solution.
3. Add 2.22 g/L sodium bicarbonate ($NaHCO_3$) to the vessel with mixing.
4. Add 5N NaOH slowly to increase the pH to 8.3-8.5. Continue mixing for 20 - 30 minutes until all the powder has dissolve.
5. Adjust pH to 7.0 by adding 5N HCL slowly.
6. Adjust to 100% of final volume with high quality purified water, such as WFI and continue to mix for 5

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minutes.

7. Measure pH and adjust pH to 7.0 with 5N NaOH or 5N HCl.
8. Adjust osmolality to 290 ± 15 mOsm/kg with a calculated amount of NaCl.
   a. Calculation formula:
      NaCl powder W (g)= VT×(290-MVOsm)/31.5
      where VT = Target volume and MVOsm = measured value of Osmolality.
9. Mix for an additional 10 minutes.
10. Sterilize immediately by membrane filtration with a pore size of 0.22 micron.
11. Label as "SagiCHO Medium".
12. Store the reconstituted medium at 2°C to 8°C with protection from light.

Cell Culture Parameters

Temperature: 37°C
Incubator atmosphere: 80% humidity, CO_2: 5-8%
Shaker speed: 110-150 rpm (amplitude: 50mm)

Cell Recovery

1. Rapidly thaw (<2 min) a vial of frozen cells in a 37°C water bath.
2. Aseptically transfer the entire contents of the vial into a 125 mL shake flask containing 30mL of pre-warmed SagiCHO™ cell culture medium.
3. Incubate shake flask at 37°C in a humified atmosphere of 5-8% CO_2 on a orbital shaker platform at 110-150 rpm.
4. Passage cells for a minimum of 2X to ensure they are fully recovered. Proceed with a normal maintenance schedule once the population doubling time remains stable.

Cell Culture Passaging

1. Aliquot pre-warmed and equilibrated SagiCHO™ cell culture medium into shake flasks.
2. Subculture when the viable cell density is ≥$1.0x10^6$ cells/mL and viability is ≥90%. Cells should be passaged when they are in mid-logarithmic growth.
3. Calculate the correct volume of cell culture and media required to inoculate a flask at a starting cell density of $0.5x10^6$ – $1.0x10^6$ cells/mL using pre-warmed SagiCHO™ medium.
4. Incubate shake flasks at 37°C in a humified atmosphere of 5-8% CO_2 on a orbital shaker platform at 110-150 rpm.
5. Passage cells by repeating the above steps every 2-3 days.

Cell Adaptation to SagiCHO™ Medium

Direct Medium Adaptation

1. Cell lines can be adapted directly from serum-free media to SagiCHO™ cell culture medium. The seeding density can be based on the passaging instructions above or determined individually.
2. Cells should be passaged several times to ensure complete adapation and optimal performance.
3. Adaptation is considered complete when the culture consistently achieve a stable viable cell density of $2x10^6$ cells/mL and a viability of ≥90% within 3-4 days, over at least 2-3 consecutive passages.

Sequential Medium Adaptation

1. The sequential adaptation method is recommended for certain cell lines that are cultured in serum-free media, in the presence of 5-10% serum or when direct adaptation results in suboptimal cell growth.
2. Monitor cell growth until the cell density reaches ≥$2.0x10^6$ cells/mL.
3. Dilute the cells using a 25:75 ratio of SagiCHO™ to the current medium.
4. Once the cells grow well in this condition, gradually increase the proportion of SagiCHO™ cell culture medium in each subsequent step, as shown in the table.
5. Adaptation is considered complete when cultures in 100% SagiCHO™ consistently achieve a stable viable cell density of $2x10^6$ cells/mL and a viability of ≥90% within 3-4 days, over at least 2-3 consecutive passages.

SagiCHO™: current medium (%)	Seeding density ($x10^5$ cells/mL)	Evaluation of cell growth	Acceptance criteria for next step
25 : 75	3 - 4	VCD & Viability	VCD ≥$2x10^6$ cells/mL, viability ≥90% over 2 passages
50 : 50	3 - 4	VCD & Viability	VCD ≥$2x10^6$ cells/mL, viability ≥90% over 2 passages
75 : 25	3 - 4	VCD & Viability	VCD ≥$2x10^6$ cells/mL, viability ≥90% over 2 passages
90 : 10	3 - 4	VCD & Viability	VCD ≥$2x10^6$ cells/mL, viability ≥90% over 2 passages
100 : 0	3 - 4	VCD & Viability	VCD ≥$2x10^6$ cells/mL, viability ≥90% over 2 passages

Cryopreservation

1. Harvest the desired quantity of cells during the mid-log pahse of cell growth, ensuring viability is above 90%.
2. Measure the VCD to confirm that the final cell density is >$1.0x10^7$ cells/mL.

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3. Prepare the freezing medium by mixing 90% SagiCHO™ cell culture medium with 10% dimethyl sulfoxide (DMSO). Cool the freezing medium to 4°C.
4. Harvest the cells by centrifuging at 400xg for 5 minutes. Remove the supernatant and resuspend the cell pellet in the cold freezing medium at a density of $>1.0x10^7$ cells/mL
5. Transfer the cell suspension to sterile cryo-vials.
6. Place the vials in a cryostorage box or a controlled rate freezing apparatus. Gradually decrease the temperature of the vials by following standard procedures (-1°C/minute).
7. For long-term storage, transfer the vials to liquid nitrogen.

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Ordering Information

Cell Culture Base Media

Name	Cat No.	Format	Pack Size
SagiCHO™ Medium	P226301	Liquid	1000mL
SagiCHO™ DPM	P220618	Dry powder	10L / 50L / 100L

Related Products:

High Performance Feeds

Name	Cat No.	Format	Pack Size
AltairCHO™ Feed	C675219	Liquid	500mL
AltairCHO™ Feed DPM	C679332	Dry powder	10L / 50L
VegaCHO™ Feed	P134305	Liquid	500mL
VegaCHO™ Feed DPM	P120826	Dry powder	10L / 50L

Highly Concentrated Feeds

Name	Cat No.	Format	Pack Size
CDFS36	C217836	Liquid	500mL / 1000mL
CDFS36 DPM	C672069	Dry powder	1L / 2L / 5L / 10L / 50L / 100L

Cell Culture Supplements

Name	Cat No.	Format	Pack Size
OPM GAL+V2 Galactosylation Enhancer	S81912	Liquid	100mL / 1000mL
OPM-ACA Anti-clumping agent	S0907001	Liquid	100mL / 500mL / 1000mL

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