

# SagiCHO™

## Chemically Defined Fed Batch Medium

— For Biomanufacturing

**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, and contains no hydrolysates, proteins, growth factors or components of unknown composition. This medium supports high level expression of recombinant proteins and therapeutic antibodies. In conjunction with OPM's new generation high performance feeds AltairCHO™ Feed or VegaCHO™ Feed, higher growth & viability, and higher expression level of the target molecule can be achieved.

### Application

SagiCHO™ cell culture medium can be used to reach & maintain high density suspension culture and to achieve high titer production in fed-batch applications. This medium is intended for large scale manufacturing of therapeutic biomolecules, as well as for research purposes, but not for human or any therapeutic use.

### Storage & Transportation

Store at 2~8°C, dark and dry  
Ship at Room temperature (Liquid), Blue ice (Dry powder)

### Shelf Life

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

### Reconstitution Method for Dry Powder

1. Measure out 90% of final required volume of purified water intended for cell culture use, e.g. WFI.  
Recommended water temperature is 25~35°C (minimum final volume  $\geq$  1L).
2. Slowly add dry powder medium at 21.73 g/L while stirring, and continue mixing for 10 minutes.  
Residual powder attached to the vessel wall should be taken into the solution.
3. Add 2.22 g/L NaHCO<sub>3</sub> to the solution, continue to stir.
4. Adjust pH to 8.3~8.5 with 5N NaOH and stir for 20~30 minutes until completely dissolved.
5. Adjust pH to 7.0 by slowly adding 5N HCl.
6. Add cell culture grade purified water to 100% final volume. Continue to stir for 5 minutes. Adjust pH to 7.0 using 5N HCl or 5N NaOH.
7. Adjust osmolality to 290 $\pm$ 10 mOsm/kg using NaCl (Calculate osmolality: required NaCl(g)= final solution volume(L) $\times$ (290 - measured value) / 31.5).
8. Continue to stir for 10 minutes. Sterile filter using a membrane filter with a pore size of 0.22 micron.

### Cell Culture Conditions

37°C, 80% humidity, 5%~8%CO<sub>2</sub>  
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. Transfer the entire contents aseptically into a 125 ml shake flask containing 30 ml prewarmed SagiCHO™ cell culture medium.
3. Incubate at 37 °C in a humidified atmosphere of 5%~8% CO<sub>2</sub> in air on a shaker (rotating at 110~130 rpm (amplitude: 50mm)).
4. Passage the cells for at least twice until fully recovered. Proceed according normal procedure after the Population Doubling Time stays stable.

## Cell Culture Passaging

1. Prewarm SagiCHO™ cell culture medium at 37 °C for 20~30min.
2. Proceed if VCD  $\geq 1 \times 10^6$ /ml & viability  $\geq 90\%$ . Cultures should be passaged during the mid-log phase.
3. Determine the correct volume of cell culture to inoculate a new flask at a starting cell density of  $0.5 \times 10^6$  ~  $1.0 \times 10^6$  cells/ml in prewarmed SagiCHO™ cell culture medium
4. Incubate flasks in a humidified 37 °C incubator with 5%-8% CO<sub>2</sub> on an orbital shaker at 110~150rpm (amplitude: 50mm).
5. Passage cells by repeating the above steps every 2~3 days.

## Medium Adaptation

### Direct Medium Adaptation

1. Cell lines may be adapted directly from serum-free media into SagiCHO™ cell culture medium. The seeding cell density can be referred to the passaging instructions or should be determined individually.
2. Cells should be passaged for a few times.
3. Adaptation is completed when the cultures attain stable VCD of  $2 \times 10^6$ /ml and viability  $\geq 90\%$  within 3~4 days over at least 2~3 passages.

### Sequential Medium Adaptation

1. For certain cell lines cultured in serum-free media, or in presence of 5~10% serum, sequential adaptation method is recommended.
2. Monitor the cell growth until the cell density has reached  $\geq 2 \times 10^6$  cells/ml.
3. Dilute the cells with a ratio of 25:75 (SagiCHO™ vs current medium), and then further dilute the culture until the cells grow well under this condition. Increase the proportion of SagiCHO™ in each subsequent operation, as is shown in the table.
4. Adaptation is completed when the cultures in 100% SagiCHO™ Cell culture medium attain stable VCD of  $2 \times 10^6$ /ml and viability  $\geq 90\%$  within 3~4 days over at least 2~3 passages.

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

## Cryopreservation

1. Harvest the desired quantity of cells in mid-log phase of growth with viability over 90%.
2. Determine VCD to ensure that the final cell density is  $> 1 \times 10^7$ /ml.
3. Prepare the freezing medium consisting of 90% SagiCHO™ Cell culture medium and 10% dimethyl sulfoxide (DMSO). Let the freezing medium cool down to 4°C.
4. Harvest cells by centrifugation at 400g for 5 minutes. Remove the supernatant and resuspend the

cell pellet with the cold freezing medium at  $>1 \times 10^7$ /ml.

5. Transfer the suspension to sterile cryo-vials.
6. Place the vials in a cryo-box or a controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
7. For long-term storage, transfer the vials to liquid nitrogen.

## Order Information

### Cell Culture Media

Name	Cat No.	Type	Volume
SagiCHO™ Medium	P226301	Liquid	1000ml
SagiCHO™ DPM	P220618	Dry powder	10L/50L/100L

### High Performance Feeds

Name	Cat No.	Type	Volume
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.	Type	Volume
CDFS36	C217836	Liquid	500ml / 1000ml
CDFS36 DPM	C672069	Dry powder	1L / 2L / 5L / 10L / 50L / 100L

### Cell Culture Supplements

Name	Cat No.	Type	Volume
OPM GAL+V2 Galatosylation enhancer	S81912	Liquid	100ml / 1000ml
OPM-ACA Anti-clumping agent	S0907001	Liquid	100ml / 500ml / 1000ml