Hyber-B100S Hybridoma Cell Serum-free Medium

Product Name: Hyber-B100S

User Manual

Contents

Description

Hyber-B100S is a serum-free culture medium developed by Shanghai Biotechnology Co., Ltd. with independent intellectual property rights, targeting the growth and metabolism characteristics of hybridoma cells. It is protein free and animal component free, suitable for high-density suspension culture of hybridoma cells, and supports efficient production of antibody.

Application

This product is intended for research or further manufacturing in the bio-manufacturing industry, but not for human or therapeutic use.

Composition

The IP rights of Hyber-B100S basal medium formulation are owned by Shanghai BioEngine Sci-Tech Co., Ltd.

This medium contains:

☑ Carbohydrates, amino acids, vitamins, bulk salts, and trace elements.

☑ 7.2 g/L D-glucose，1 g/L P188, 8 mM glutamine.

Not contain:

☒ Antibiotics, HEPES and phenol red.

☒ Raw materials from animal sources.

Storage

- Store medium at 2-8℃, away from light.
- Once opened, the powder medium should be stored protected from moisture in a tightly sealed container.
- Do not use it after the expiration date or being damped.

Reconstitution of Powder Medium

Table 1 shows the preparation of Hyber-B100S medium [1].

Ingredients	Concentration
Hyber-B100S medium powder	23.91 g/L [2]
Recombinant human insulin	10.00 mg/L
Hyber-B100S Additive	1 mL/L
Sodium bicarbonate	2.00 g/L

Table 1. Preparation of Hyber-B100S medium

1) Weigh 100% water of the final volume into the preparation container using pure water, ultrapure water, or water for injection at 20-30℃. Mix thoroughly (Power per Volume (P/V)>10 W/m^3) without creating air bubbles.

2) Accurately weigh the corresponding mass of Hyber-B100S medium powder at a concentration of 23.91 g/L, add it into the preparation container of 1) step, and stir well for 20-30 minutes.

3) Weigh 10 mg/L of recombinant human insulin, dissolve it with 10 mmol/L dilute hydrochloric acid on the day of use, and add it into the preparation container of 1) step.

   P.s.: Insulin should be prepared and used immediately.

4) Slowly adjust to pH 6.0-6.5 with 5-10 mol/L sodium hydroxide solution. Stir for 10-20 minutes. At this point, the solution should be clear.

5) Add 1 mL/L Hyber-B100S Additive near the liquid level in the container.

6) Weigh 2.00 g/L of sodium bicarbonate powder, add it slowly near the liquid level in the container. Stir for 10-20 minutes.

7) Adjust to pH 7.0-7.4 with sodium hydroxide or hydrochloric acid solution.

8) Pass the medium solution through a pore size of 0.22 or 0.2 μm sterile filter membrane, such as PES, using a pulse pump or compressed air (3-15 psi).

9) Use the prepared medium liquid immediately or store it in glass bottles, PET storage bottles, or disposable storage bags with an oxygen barrier membrane in a dark environment of 2~8℃. It's recommended for use within 1 month.

Note:

[1] The above parameters (such as stirring time and P/V) are set for small-scale liquid preparation. Adjust these parameters for large-scale preparation based on container capacity to ensure full dissolution of dry powder.

[2] The "g/L" unit denotes volumetric concentration (solute mass/water volume).

Specifications of final liquid medium

Test	Unit	Specification
pH		7.0 – 7.4 [3]
Osmolality	mOsm/kg	300 – 360
Turbidity	NTU	< 4.00

Table 2. Specifications of final liquid medium

Note:

[3] The pH buffer system of the product is carbon dioxide-sodium bicarbonate. The final pH value should be strictly controlled within the specific range outlined in Table 2. The following operations, such as prolonged reconstitution time or aeration in the bioreactor without pH control, can result in a gradual pH increase. There is a risk of metal ion precipitation when the pH value exceeds the upper limit.

Cryopreservation

1) Harvest cells in the mid-log phase of growth with >90% viability by centrifugation at 190×g for 5 minutes.

2) Prepare cryopreservation medium with 93% Hyber-B100S medium and 7% DMSO on the day of use.

3) Resuspend cells in cryopreservation medium to a final viable cell density of 2.5-3.5×10^7 cells/mL or as required.

4) Dispense aliquots of the cell suspension into cryovials.

5) Achieve cryopreservation in an automated or manual controlled rate freezing apparatus (0.5-1℃ decrease per minute is suggested).

6) Transfer frozen cells to liquid nitrogen storage.

Cell Recovery

1) Rapidly thaw frozen cells in a 37℃ water bath. Transfer to a clean workbench as soon as melted or with small ice crystals.

2) Transfer the vial content to a centrifuge tube containing 10 mL of prewarmed Hyber-B100S medium. Harvest the cells by centrifugation at 190×g for 5 minutes and discard the supernatant.

3) Resuspend cells by prewarmed Hyber-B100S medium to a viable cell density of 0.8-1.2×10^6 cells/mL in a 125 mL shake flask.

4) Incubate the shake flask at 37℃ in a humidified atmosphere of 5% CO_2 in air on an orbital shaker platform rotating at 110-130 rpm (110 rpm for 50 mm amplitude; 130 rpm for 10 mm amplitude).

5) Cells should be sub cultured and adapted at least two passages. After the cell specific growth rate (or doubling time) reaches stability, subsequent operations can be carried out.

Subculture Cells

1) Ensure that the cell viability is >90%, and the growth rate is in mid-logarithmic phase prior to subculturing.

2) Calculate the volume of cell culture and prewarmed medium necessary to seed at 0.8-1.2×10^6 viable cells/mL in a shake flask.

3) Incubate at 37℃ in a humidified atmosphere of 5% CO_2 in air on an orbital shaker platform rotating at 110-130 rpm (110 rpm for 50 mm amplitude; 130 rpm for 10 mm amplitude).

4) Subculture cells every two days according to the above steps.

Related Product

Product	Cat. No.	Form	Size	Packaging	Notes
Hyber-B100 Hybridoma Cell Serum-free Medium	EXP0118901	Liquid	1 L	Bottle	● SF, PF, ADCF ● Suitable for high-density hybridoma cell suspension cultures and supports high protein expression
Hyber-B100S Hybridoma Cell Serum-free Medium	EXP0111201	Powder	100 L	Bag	
	EXP0111202	Powder	10 L	Bag	
	EXP0111203	Powder	5 L	Bag	
Hyber-F100S Hybridoma Cell Serum-free Feed Medium	EXP0111301	Powder	20 L	Bag	
	EXP0111302	Powder	2 L	Bag	

Scan the QR code for more product information.

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