Recommended Protocol for Testing JSBio CHO media

JSBio CHO Base media & CHO Feed media

JSBio CHO base media and feed media are chemically defined media, and do not contain animal components, proteins, hydrolysates and other uncertain components. These nutritionally balanced media not only can support the high-density growth of CHO cells and maintain high cell viability, but also can meet the requirements of the fed-batch process for high-expression of proteins with satisfactory quality.

Storage & Stability

The dry powder media and liquid media should be stored at 2–8°C and protected from light. Under this condition, the dry powder media are stable for a minimum of two years and the liquid media for a minimum of 6-months. Addition of other additives may affect the shelf life of the media. If the liquid media show deterioration such as color change, precipitation, or cloudiness, please stop using and contact your local sales representative for assistance. An aseptic technique should be used when handling or supplementing these media.

Culture Conditions

Culture Type: Suspension

Culture Vessel: TPP tubes/ Shaker flask/ Bioreactor

Shaking Speed: For TTP tubes cultivation, it is recommended 200 rpm @50 mm orbital diameter. For shake flasks cultivation, it is recommended 125–135 rpm @25 mm orbital diameter or 80–100 rpm @50mm orbital diameter.

Incubator Atmosphere: 37°C, 5% CO_2, 80% relative humidity.

Recover Cell

1. Quickly thaw (<1 minute) the fluids in the cryovial in a 37°C water bath.
2. Transfer all the cell liquid to a 50 mL sterile centrifuge tube containing 10 mL prewarmed CD CHO base media, centrifuge at 200 × *g* (about 800 rpm) for 5 minutes, discard the supernatant and use 10 mL prewarmed CD CHO base media for resuspension, and inoculate the cells by seeding at the density of 0.8–1.2 × 10^6 cells/mL.
3. Cultivate the cells according to “Culture Conditions”.

Cell Passage and Scale-up

1. Carry out the cell passage and scale-up after the cells have been recovered for 72 ± 6 hours. Ensure that the cells are in mid-logarithmic phase when conduct cell passage and scale-up. Determine the viable cell density (× 10^6 cells/mL) and cell viability (%) before starting the process.
2. Calculate required volume of CD CHO media and the cell culture necessary to seed a TPP tube or shake flask at the density of 0.8–1.2 × 10^6 cells/mL.
   Note: If cell density does not reach 4 × 10^6 cells/mL before passage, centrifuge cells at 200 × *g* (about 800 rpm) for 5 minutes, discard the supernatant and resuspend the cell pellet in freshly prepared CD CHO base media.
3. Cultivate the cells according to “Culture Conditions”. It is recommended to carry out 3–5 continuous passages prior to conducting subsequent experiments.

Adapt CHO cell to CD CHO Base Media

We recommend direct adaptation as well as sequential adaptation. Different cells will behave differently. It is critical that the cell viability is ≥90% and the growth rate is in mid-logarithmic phase prior to initiating an adaptation procedure.

Direct Adaptation

1. For direction adaptation of CHO cells grown in other serum-free media into the JSBio CD CHO base media, dilute cells into 100% JSBio CD CHO base media using a centrifugation method when transferring for the first time.
2. Centrifuge cells at 200 × *g* (about 800 rpm) for 5 minutes, discard the supernatant and resuspend the cell pellet in

freshly prepared CD CHO base media at the seeding density of 0.8–1.2 × 10^6 cells/mL.

3. Cultivate the cells according to “Culture Conditions” and refer to the "Cell Passage and Scale-up" for cell subculture until consistent growth is achieved.

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

Sequential Adaptation

1. According to the seeding density of 0.8–1.2 × 10^6 cells/mL, gradually transfer the cells to the media mixed with the original growth media and the JSBio CD CHO base media in different ratios (90 : 10, 75 : 25, 50 : 50, 25 : 75, 0 : 100), cultivate the cells according to "Culture Conditions".
2. When adapting to each mixed media, refer to "Cell Passage and Scale-up" for subculture. Generally, each stage needs to be passaged for 2–3 times, which can be adjusted according to the growth state of the cells.
3. Adaptation is considered successful until cells can grow stably in 100% CD CHO base media.

Cryopreserve Cells

It is recommended to use cells in mid-logarithmic phase with cell viability >90% for cryopreservation.

1. Determine the viable cell density (× 10^6 cells/mL), and calculate the required volume of cryopreservation media and cell culture to give a cryopreserve cell density of 1 × 10^7 cells/mL and cryopreserve volume of 1 mL per tube.
2. Prepare the required volume of cryopreservation media of 90% CD CHO base media + 10% DMSO and store at 2–8°C until later use.

   Note: Prepare cryopreservation media on the day of use.
3. Harvest cells by centrifugation at 200 × *g* (about 800 rpm) for 5 minutes, and resuspend the pellet in the pre-determined volume of 2–8°C cryopreservation media.
4. Aliquot the cell suspension into cryopreservation tubes and immediately transfer it to a pre-cooled programmed cooling box. After the programmed cooling, transfer it to a liquid nitrogen tank for long-term storage.

Fed-batch Culture

After the cells are adapted to JSBio CD CHO base media and serially passaged for 3–5 times, they can be cultured in Fed-batch. On the day of passage, seed the cells into CD CHO Base media at the seeding density of 0.8–1.2 × 10^6 cells/mL. The following is the feeding strategy of CD feed media.

Base Media	Feed and Feeding Strategy
CD CHO 011 CD CHO 012 CD CHO 031 CD CHO 032 CD CHO 038 CD CHO 041 CD CHO 043 CD CHO 045 CD CHO 047 CD CHO 050 CD CHO 051	**CD Feed 002:** Add 4% each time on day 3, 5, 7, 9, 11, 13
	CD Feed 008: Add 4% each time on day 3, 5, 7, 9, 11, 13 **CD Feed 009:** Add 0.4% each time on day 3, 5, 7, 9, 11, 13
	CD Feed 008.2: Add 4% each time on day 3, 5, 7, 9, 11, 13 **CD Feed 009:** Add 0.4% each time on day 3, 5, 7, 9, 11, 13
	CD Feed 018: Add 4% each time on day 2, 4, 6, 8, 10, 12 **CD Feed 017:** Add 0.4% each time on day 2, 4, 6, 8, 10, 12
	CD Feed 018: Add 2.5%–3.5% each day from day 2 **CD Feed 017:** Add 0.25%–0.35% each day from day 2
	ALLY CHO 100a: Add 4% each time on day 3, 5, 7, 9, 11, 13 **ALLY CHO 100b:** Add 0.4% each time on day 3, 5, 7, 9, 11, 13
	CD Feed 020: Add 4% each time on day 2, 4, 6, 8, 10, 12 **CD Feed 017:** Add 0.4% each time on day 2, 4, 6, 8, 10, 12

Note:

1. It is recommended to set up two replicates for each condition.
2. Glucose should be controlled at 6 g/L. The critical concentration needed for the glucose addition is 3–4 g/L.
3. The volume of feed media and glucose added can be calculated according to the actual working volume of the day.