Recommended Protocol for Testing APEX CHO 100

CHO Perfusion Media

JSBio CHO Perfusion media are chemically defined media, and do not contain animal components, proteins, hydrolysates, and other uncertain components. JSBio CHO Perfusion media are nutrient-concentrated media that can provide enough nutrients for maintaining high cell density growth (VCD) and high protein productivity at lower media exchange rates, which is 1–2 vessel volume per day (VVD).

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 for Cell Passage and Scale-up

Culture Type: Suspension

Culture Vessel: TPP tube/ Shaker flask

Shaking Speed: For TTP tube 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.

Cell Culture Media: APEX CHO 100 is a nutrient-concentrated media that is only used in the perfusion culture process. And JSBio CHO base media can used for cell passage and scale-up, e.g. CD CHO 031 (88031-585), CD CHO 041 (88041-1330), CD CHO 045 (880045-1509).

Recover Cells

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 JSBio CHO base media, centrifuge at 200 × *g* (about 800rpm) for 5 minutes, discard the supernatant and use 10 mL prewarmed JSBio CHO base media for resuspension and seed the cells 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 JSBio CHO base 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 4.0 × 10^6 cells/mL before passage, centrifuge cells at 200 × *g* (about 800rpm) for 5 minutes, discard the supernatant and resuspend the cell pellet in freshly prepared JSBio 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 JSBio 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 CHO base media, dilute cells into 100% JSBio CHO base media using a centrifugation method when transferring for the first time.
2. Centrifuge cells at 200 × *g* (about 800rpm) for 5 minutes, discard the supernatant and resuspend the cell pellet in freshly prepared JSBio 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 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% JSBio 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% JSBio 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 800rpm) 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.

Pseudo-perfusion Culture

Culture Conditions for Perfusion Culture:

Culture Vessel: TPP tube/ Shaker flask

Initial Working Volume: 10 mL for 50 mL TPP tube, 20 mL for 125 mL shaker flask, 35 mL for 250 mL shaker flask

Shaking Speed: 220 rpm for TPP tube and 120–130 rpm for shaker flask. Please tilt the culture vessel to 45° for better dissolved oxygen conditions. The shelves of TPP tubes can be tilted directly. For those using a shaker flask as a culture vessel, the shaker flask with baffle can be used.

Incubator Atmosphere: Using the temperature of 37°C from Day 0 to Day 2, and shift the temperature to 33℃ from Day 2 to the end. 5% CO_2, 80% relative humidity.

Perfusion Strategy:

1. The cells are passaged and scaled-up in JSBio CHO base media, on the day of passage, centrifuge cells at 200 × *g* (about 800rpm) for 5 minutes, discard the supernatant and resuspend the cell pellet in freshly prepared APEX CHO 100 media with the density of 9.0–11.0 × 10^6 cells/mL.
2. Centrifuge, and resuspend the cells with freshly prepared APEX CHO 100 media every day for Pseudo-perfusion culture. Determine the viable cell density (× 10^6 cells/mL) and cell viability (%) before centrifugation and determine the metabolic parameters with the supernatant. The recommended perfusion volume is 1–1.5VVD.

Note:

1. **It is recommended to set up two replicates for each condition.**
2. **Glucose should be controlled at 6g/L. The critical concentration needed for the glucose addition is 3–**

4g/L.

3. **The volume of glucose added can be calculated according to the actual working volume of the day.**