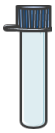
Thaw Frozen XtenCHO™ Cells



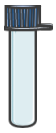
90 sec
37°C

1 Incubate for 90 sec max. at 37°C

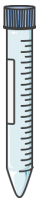
Remove the vial of cells from liquid nitrogen and swirl in a 37°C water bath for 90 seconds maximum until only a small amount of ice remains.

2 Decontaminate with 70% EtOH

Decontaminate the vial by wiping it with 70% ethanol before opening it in a laminar flow hood.



XtenCHO™ Expression Medium



3 Transfer 8 mL of XtenCHO™ Expression Medium with 8 mM L-Glutamine

Transfer the entire contents of the cryovial into 8 mL of pre-warmed XtenCHO™ Expression Medium supplemented with supplemented with 8 mM L-Glutamine. Rinse the cryovial with XtenCHO™ Expression Medium. Mix by inversions.

4 300 x g 5 min

Centrifuge the cellular suspension for 5 minutes at 300 x g. Discard the supernatant.

5 Transfer XtenCHO™ Expression Medium with 8 mM L-Glutamine and 0.5% Anticlumping agent

Resuspend the XtenCHO™ Cells in a small volume of XtenCHO™ Expression Medium supplemented with 8 mM L-Glutamine. Determine viable cell number and viability. Transfer the appropriate number of cells to seed 30mL of pre-warmed XtenCHO™ Expression Medium supplemented with 8 mM L-Glutamine, in a 125-mL disposable, sterile, vent-cap Erlenmeyer shaker flask. Add 0.5% Anticlumping agent.

6 Incubate at 37°C, 5% CO_2 >80% humidity under agitation

Incubate the cells in a 37°C incubator with ≥80% relative humidity and 5% CO_2 on an orbital shaker platform.

37°C

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Subculture XtenCHO™ Cells

1 Determine VCD and calculate seeding

Determine the viable cell density (VCD) of the culture and calculate the volume of cell suspension required to seed a new shake flask.

2 300 x g 5 min

Centrifuge the cellular suspension for 5 minutes at 300 x g. Discard the supernatant.

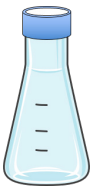
XtenCHO™ Expression Medium

3 Transfer 8 mL of XtenCHO™ Expression Medium with 8 mM L-Glutamine and 0.5% Anticlumping agent

Resuspend cells into fresh, pre-warmed XtenCHO™ Expression Medium supplemented with 8 mM L-Glutamine, and transfer cells in a baffled shake flask. Add Anticlumping agent to a final concentration of 5%.

4 Incubate at 37°C, 5% CO_2 >80% humidity under agitation

Incubate flask in a 37°C incubator with ≥80% relative humidity and 5% CO_2 on an orbital shaker platform until cultures reach a density of $1.5 \times 10^6 – 2.5 \times 10^6$ viable cells/mL.



37°C

Repeat **Steps (1) – (4)** to maintain and amplify cells for transfection or cryopreservation.

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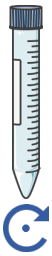
Crypreserve XtenCHO™ Cells

1 Culture cells to reach densities of $1.5 \times 10^6 – 2.5 \times 10^6$ cells/mL

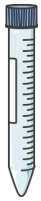
Allow cells to reach a viable cell density of $1 \times 10^6 – 2 \times 10^6$ cells/mL and >95% viability before cryopreservation.

2 300 x g 5 min

Centrifuge the desired number of cells at 300 × g for 5 minutes to pellet, discard the spent medium.



Freezing Medium

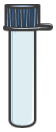


3 Dilute cells at in freezing medium at 1×10^7 cells/mL

Dilute cells to a final density of 1×10^7 viable cells/mL with freezing medium. Gently resuspend the cell pellet by pipetting. Freezing medium : XtenCHO™ Expression Medium (supplemented with 8 mM L-Glutamine and 0.5% Anticlumping agent) with 10% DMSO.

4 Aliquot 1 mL per cryovial

Aliquot 1 mL of cell suspension per cryovial.



5 Freeze at -80°C
Store in liquid nitrogen

Place cryovials quickly in a freezing container designed to achieve a rate of cooling of -1°C/minute, the optimal rate for cell preservation. Transfer frozen vials to liquid nitrogen for long-term storage.

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XtenCHO™

Transfect XtenCHO™ Cells

PART I

Day -1: Split Cells

1. Split the cells to reach 1.2 x 10^6 cells/mL
Incubate at 37 °C, 5% CO_2 for 24 h with agitation

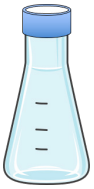
Split the XtenCHO™ culture to a final density of 1.2 × 10^6 viable cells/mL in XtenCHO™ Expression Medium supplemented with 8 mM L-Glutamine but without Anticlumping agent. Allow the cells to grow overnight.

Day 0: Transfection

2 Determine the VCD and viability

Determine viable cell density (VCD) and viability.

XtenCHO™ Expression Medium

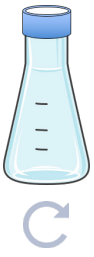


3 Seed cell with XtenCHO™ Expression Medium containing 8 mM L-Glutamine

Seed 5 × 10^6 viable cells/mL with half of the final volume wanted of fresh XtenCHO™ Expression Medium supplemented with 8 mM L-Glutamine.

4.1 Mix plasmid DNA by inversion

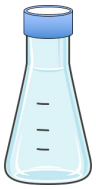
Start the preparation of plasmid DNA and XtenCHO™ Reagent (Working solution previously prepared from Stock solution) by mixing the plasmid by inversion.



4.2 Add DNA (in a row) & swirl to mix

Add the plasmid DNA directly on the cells in a row. Gently swirl the flasks to mix.

XtenFect Reagent



4.3 Add the XtenFect Reagent

Mix the XtenFect Reagent by inversion and add it drop-by-drop on the cells, gently swirling the flask during addition.

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XtenCHO™

Transfect XtenCHO™ Cells

PART II

Day 0: Transfection

2 h
37°C

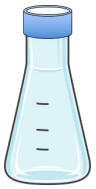
5 Incubate 2 h at 37°C, 5% CO_2 >80% humidity with agitation

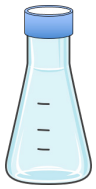
Incubate the cells in a 37° C incubator with a humidified atmosphere of 5% CO_2 in air on an orbital shaker for 2 hours.

6 Add XtenCHO™ Enhancer and Expression Medium with 8 mM L-Glutamine

2 hours post-transfection, add the XtenCHO™ Enhancer directly on the cells. Then, add the other half of media supplemented with 8 mM L-Glutamine to complete the procedure.

XtenCHO™ Enhancer



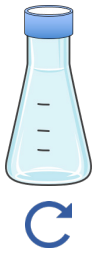


24 h
37°C

7 Incubate for 24 h at 37°C, 5% CO_2 >80% humidity with agitation

Start the preparation of plasmid DNA and XtenCHO™ Reagent (Working solution previously prepared from Stock solution) by mixing the plasmid by inversion.

Day 1: Add Anticlumping agent and shift Temperature

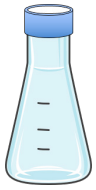


8 Add Anticlumping agent (swirling) Shift temperature 37°C -> 33°C

Add anticlumping agent to final concentration of 5% the flask, gently swirling the flask during addition.

9 Incubate for 10-15 d at 33°C, 5% CO_2, >80% humidity and agitation

Incubate the cells in a 33°C incubator with a humidified atmosphere of 5% CO_2 with shaking. Check viability regularly and harvest when viability drops under 50% or at Day 15.



10-15 d
33°C

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Guidelines for Culture and Transfection of XtenCHO™ Cells

Table 1. Recommended seeding densities for routine subculturing.

Sub-culture timing	Recommended seeding density
For cells ready 2 days post-subculture	0.3 x 10^6 viable cells/mL
For cells ready 3 days post-subculture	0.2 - 0.3 x 10^6 viable cells/mL

Table 2. Recommended volumes and shaking speed for routine cell culture maintenance.

Flask size	125 mL	250 mL	500 mL	1000 mL	2000 mL	3000 mL
Culture volume (mL)	30-40	60-100	125-200	250-400	500-800	750-1200
Shake speed	140 ± 10 rpm (19-mm shaking diameter)					95 ± 5 rpm
	130 ± 5 rpm (25-mm shaking diameter)					90 ± 5 rpm
	95 ± 5 rpm (50-mm shaking diameter)					85 ± 5 rpm
Flask type	Vented, baffled					

Table 3. Guidelines for cell number and volumes for transfection at different scales.

Cell culture vessel	Cell number (x10^6)	DNA quantity (g)	XtenFect Reagent, Working Solution (µL)	XtenCHO™ Enhancer (L)	Transfection volume (mL)	Final transfection volume (mL)
125 mL flask	75	48	144	96	15	30
250 mL flask	200	128	384	256	40	80
500 mL flask	500	320	960	640	100	200
1000 mL flask	625	400	1200	800	125	250
2000 mL flask	1250	800	2400	1600	250	500
3000 mL flask	2500	1600	4800	3200	500	1000

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