WATERPROOF

Thaw Frozen XtenCHO™ Race Cells

90 sec
37°C

1 Incubate the cryovial for 90 sec max. at 37°C

Remove the vial of cells from the 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 the cells into 8 mL of XtenCHO™ Expression Medium supplemented with 8 mM L-Glutamine

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

4 300 x g 5 min

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

5 Seed the cells with XtenCHO™ Expression Medium containing 8 mM L-Glutamine

Resuspend the XtenCHO™ Race 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 30 mL of pre-warmed XtenCHO™ Expression Medium supplemented with 8 mM L-Glutamine, in a 125-mL disposable and sterile vent-cap Erlenmeyer shaker baffled flask. Seed the cells at a density of 0.3 x 10^6 cells/mL, as recommended in **Table 1**. Add the Anticlumping agent at a final concentration of 0.5%.

6 Incubate the cells at 37°C

Incubate the cells in a 37°C incubator with ≥80% relative humidity and 5% CO_2 on an orbital shaker platform. See recommended shaking speed in **Table 2**.

37°C

For more information visit
https://www.proteogenix.science/product-category/xtencho-transient-expression-system/

XtenCHO™ RACE

Cryopreserve XtenCHO™ Race Cells

1

Culture the cells to reach densities of 1.5 x 10^6 – 2.5 x 10^6 cells/mL

Allow the cells to reach a viable cell density of 1.5 × 10^6 – 2.5 × 10^6 cells/mL and >95% viability before cryopreservation.

300 x g 5 min

2

Centrifuge the desired number of cells at 300 × g for 5 minutes, discard the supernatant.

Freezing Medium

3

Resuspend the cells in freezing medium at 1 x 10^7 cells/mL

Resuspend the cells at a final density of 1 × 10^7 viable cells/mL in 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.

Aliquot 1 mL per cryovial

4

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 and freeze at -80°C.

Transfer frozen vials to liquid nitrogen for long-term storage.

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XtenCHO™ RACE

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Subculture XtenCHO™ Race Cells

1 Determine the VCD and calculate seeding density

Determine the viable cell density (VCD) of the culture and calculate the volume of cell suspension required to seed a new shake baffled flask. Recommended seeding densities are indicated in **Table 1.**

2 300 x g 5 min

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

XtenCHO™ Expression Medium

3 Seed the cells with XtenCHO™ Expression Medium supplemented with 8 mM L-Glutamine

Resuspend the cells in fresh, pre-warmed XtenCHO™ Expression Medium supplemented with 8 mM L-Glutamine, and transfer them into a baffled shake flask. Add the Anticlumping agent at a final concentration of 0.5%.

4 Incubate the cells at 37°C

Incubate the 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.

For more information visit

https://www.proteogenix.science/product-category/xtencho-transient-expression-system/

XtenCHO™ RACE

Guidelines for Subculturing and Transfecting XtenCHO™ Race Cells

Table 1. Recommended seeding densities for routine subculturing.

Subculture 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 speeds for routine cell culture maintenance.

Flask size	24 Deepwell plate	125 mL	250 mL	500 mL	1000 mL	2000 mL	3000 mL
Culture volume (mL)	3.5	30-40	60-100	125-200	250-400	500-800	750-1200
Shaking speed							
19-mm orbit	275 ± 5 rpm	140 ± 10 rpm					95 ± 5 rpm
25-mm orbit	240 ± 5 rpm	130 ± 5 rpm					90 ± 5 rpm
50-mm orbit	x	95 ± 5 rpm					85 ± 5 rpm
Vessel type	Deepwell plate	Flask, vented and baffled					

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

Cell culture vessel	Cell number (x10^6)	DNA quantity (µg)	XtenFect Reagent, Working Solution (µL)	XtenCHO™ Race Enhancer (µL)	Transfection volume (mL)	Final transfection volume (mL)
24 Deepwell plate	8.75	5.6	16.8	11.2	1.75	3.5
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

WATERPROOF

Transfect XtenCHO™ Race Cells

PART 1

DAY -1: SPLIT THE CELLS

Seed the cells at a density of 1.2 x 10^6 cells/mL. Incubate at 37°C, 5% CO_2 for 24 h with shaking

Split the XtenCHO™ Race culture to a final density of 1.2 × 10^6 viable cells/mL in XtenCHO™ Expression Medium supplemented with 8 mM L-Glutamine. **Do not add Anticlumping agent at this step.** Allow the cells to grow for 24 hours.

DAY 0: TRANSFECTION

2

Determine the VCD and viability

Determine the viable cell density (VCD) and viability.

Seed the cells in XtenCHO™ Expression Medium containing 8 mM L-Glutamine

3

+ XtenCHO™ Expression Medium

Seed 5 × 10^6 viable cells/mL with **half** of the final required volume of fresh XtenCHO™ Expression Medium supplemented with 8 mM L-Glutamine without Anticlumping agent.

4.1

Prepare the reagents for transfection

Prepare the Working solution of XtenFect Reagent from the Stock solution, and mix by inversion. Mix the plasmids by inversion.

Add DNA (in a row) & swirl to mix

4.2

+ DNA

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

+ XtenFect Reagent

4.3

Add the XtenFect Reagent

Mix the XtenFect Reagent by inversion and add it drop-by-drop to the cells. Gently swirl the flask during this process.

For more information visit

https://www.proteogenix.science/product-category/xtencho-transient-expression-system/

XtenCHO™ RACE

Transfect XtenCHO™ Race Cells

PART II

DAY 0: TRANSFECTION

2 h
37°C

5 Incubate the cells for 2 h at 37°C

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

XtenCHO™ Race Enhancer
+
Expression Medium

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

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

24 h
37°C

7 Incubate the cells for 24 h at 37°C

Incubate the cells for 24 hours in a 37°C incubator, 5% CO_2, and >80% humidity, with shaking.

DAY 1: ADD THE ANTICLUMPING AGENT AND SHIFT TEMPERATURE

+ Anticlumping agent

8 Add the Anticlumping agent

Add the Anticlumping agent at a final concentration of 0.5%. Gently swirl the flask.

Shift
37°C → 33°C

10–14 days
33°C

9 Incubate the cells in flask for 10–14 days at 33°C*

Shift the temperature from 37°C to 33°C. Incubate the cells in a 33°C incubator with a humidified atmosphere, 5% CO_2, with shaking. Check the viability regularly and harvest once it drops below 50% or at Day 15 post-transfection.

*in 24 Deepwell plate, incubate the cells for 7 days at 33°C.

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