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EX-PRESS 293™ Cell Lines

For use with EX-PRESS 293™ and EX-PRESS 293™-BL Cells
Catalog Numbers: MC001, MC001P, MC002, and MC002P
Version: A1.1 – April 2025
Limited Use & License

Product Information

Contents:

Cryovials of frozen cells containing a total of 1 x 10^7 cells in 1 ml of freezing medium (BalanCD HEK293 supplemented with 10% DMSO). Cells are available as single vials, or in packs of 6 vials, see below.

PRODUCT	QUANTITY SUPPLIED	CATALOG NUMBER
EX-PRESS 293™	1 vial 6 vials	MC001 MC001P
EX-PRESS 293™-BL	1 vial 6 vials	MC002 MC002P

Storage: The cells are shipped on dry ice. Upon receipt, cryovials should be thawed and used immediately, or transferred to storage in liquid nitrogen. Contact support@abdesignlabs.com if the cells are not frozen upon arrival.

Quality control: The cells were certified to meet all specifications, including absence of Mycoplasma species by qPCR screen.

Product Description

EX-PRESS 293™ is a HEK293 cell line adapted to be maintained in serum-free media and suspension culture. **EX-PRESS 293™** cells have been optimized for high density, transfection efficiency and recombinant protein production using a protocol designed to reduce transfection requirements without sacrificing recombinant protein production or quality.

EX-PRESS 293™-BL is a derivative of the **EX-PRESS 293™** cell line that has been modified for highly efficient in cellulo biotinylation of recombinant proteins that contain an Avi-tag™.

Required Reagents Not Supplied

- Sterile 125 ml Optimum Growth® Shake Flasks (Thomson, Cat#931110)
- BalanCD HEK293 (Fujifilm Irvine Scientific, Cat# 91165)
- BalanCD HEK293 Feed (Fujifilm Irvine Scientific, Cat# 91166)
- L-glutamine, 200 mM (any supplier)
- D-biotin, 50 mM (**EX-PRESS 293™-BL** only, any supplier)
- Zeocin®, 100 mg/ml solution, (Optional, **EX-PRESS 293™-BL** only, InvivoGen, Cat#ant-zn-05)

Protocols

General Cell Culture

Handling

All reagents and equipment that come in contact with the cells should be sterile. Work with the cells should be done in a laminar flow hood using proper aseptic technique. Vigorous shaking or pipetting when working with the cells should be avoided to maintain cell health.

Cell Thawing

1. Add 30 ml of BalanCD HEK293 supplemented with 2 mM L-glutamine pre-warmed to 37°C in a 125 ml Optimum Growth® shake flask.
2. Transfer a vial of **EX-PRESS 293™** cells from storage in liquid nitrogen to a 37°C water bath and swirl gently for 60 seconds to rapidly thaw the cells. Do not submerge the vial.
3. Before the cells have completely thawed, remove the vial from the water bath and spray the vial thoroughly with 70% ethanol to decontaminate the vial exterior before opening the vial in a laminar flow hood.

   Note: Incubating the cells too long at 37°C will result in loss of viability.

4. Gently mix the contents of the vial and transfer to the flask prepared previously.
5. Transfer the shake flask into a shaking, humidified incubator set to 37°C, 8% CO_2 and 150 rpm (20 mm orbital diameter).
6. Estimate concentration of cell suspension three to four days post-thaw by hemocytometer or automated cell counter. Viability should be ≥ 90% with a density ≥ 1 x 10^6 cells/ml. Continue incubation if needed, or proceed to routine subculturing.

Cell Subculturing

EX-PRESS 293™ cells have a doubling time of approximately 24 h during log phase growth. Cells should be passaged every 3 to 4 days to maintain density of ≤ 3 x 10^6 viable cells per ml.

1. Estimate concentration of viable cells in suspension by hemocytometer or automated cell counter, and calculate volume required for seeding a new flask at 3 x 10^5 viable cells per ml.

   Note: We recommend using Optimum Growth® shake flasks for culturing. The unique flask design allows for high volume in a small form factor suitable for our recommended bioprocessing method while maintaining the same shake conditions across flasks of all sizes (125 ml to 7 L).

2. Transfer calculated volume of cell suspension to fresh, pre-warmed, BalanCD HEK293 media supplemented with 2 mM L-glutamine.

   Optional: EX-PRESS 293™-BL cells are resistant to Zeocin® and can be cultured with media supplemented with 100 µg/ml Zeocin® to ensure stable integration of biotin ligase is maintained.

3. Incubate flasks in a shaking, humidified incubator set to 37°C, 8% CO2 and 150 rpm (20 mm orbital diameter).

4. Repeat steps 1-3 to maintain or expand cells. We recommend discarding cultures after 30 passages.

Cryopreservation

1. Estimate cell density and culture volume required to preserve the desired number of 1 ml aliquots containing 1 x 10^7 cells per ml.

2. Expand culture up to required volume while maintaining densities ≤ 3 x 10^6 cells/ml and ≥ 90% viability.

3. Pellet cells by centrifugation at 300 x *g* for 5 min, aspirate spent medium.

4. Resuspend cell pellet in BalanCD HEK293 media supplemented with 2 mM L-glutamine and 10% DMSO pre-chilled to 4°C at a final concentration of 1 x 10^7 cells/ml.

5. Dispense 1 ml of cell suspension into each cryogenic vial. Place vials in an insulated container and store at -80°C overnight.

6. Transfer vials to liquid nitrogen vapor phase for long-term storage.

Recombinant Protein Production

Transfection

EX-PRESS 293™ cells have been optimized for commercially available cationic-lipid transfection systems but are also compatible with other methods of transfection (e.g., PEI). We recommend researchers follow the manufacturer's protocol for the desired method and reagent.

We recommend testing transfection conditions with a fluorescent reporter plasmid first (i.e., **TGEX™-eGFP-Zeo**, Cat# MX022) to evaluate transfection efficiency and determine optimal transfection conditions.

TGEX™ series mammalian expression vectors have been optimized for recombinant antibody production in **EX-PRESS 293™** cells, see *Related Products* for available formats and isotypes.

Fed-batch production

The following protocol is the recommended bioprocessing method using **EX-PRESS 293™** cells.

1. Subculture and expand **EX-PRESS 293™** cells until density reaches ≥3 x 10^6 cells/ml in half of the total desired production volume (*Transfection volume*).

   Note: Do not use cultures with densities ≥ 3 x 10^6 cells/ml for routine maintenance.

2. On the day of transfection, seed **EX-PRESS 293™** cells at 3 x 10^6 cells/ml in the transfection volume in an Optimum Growth® flask with a fill volume suitable for the desired production volume.

3. Transfect culture with 1 µg/ml transfection-grade mammalian expression vector according to the protocol of preferred transfection reagent.

   Note: For expression of proteins that require co-transfection (e.g., antibody heavy and light chain vectors) we recommend evaluating the ratio of the vectors while maintaining the total DNA concentration at 1 µg/ml.

   Important: Supplement the media of **EX-PRESS 293™-BL** fed-batch productions with 50 µM D-biotin to facilitate in cellulo biotinylation.

4. Return culture to incubate at 37°C and 8% CO_2 with shaking at 150 rpm (20 mm orbital diameter).

5. If required, enhance cultures with components of the preferred transfection reagent system at times specified in manufacturer's protocol the day after transfection.

6. Two days post-transfection, double the volume of the culture to the final production volume using pre-warmed BalanCD HEK293 supplemented with 2 mM L-glutamine and 50 µM D-biotin (**EX-PRESS 293™-BL** only).

   Note: Optimum Growth® flasks are recommended as the transfection volume can be doubled to the final production volume in the same flask. Other flasks may be used provided the production volume does not exceed the recommended flask fill volume to maintain optimal gas exchange.

7. Continue to incubate cells, feeding with BalanCD HEK293 Feed as desired according to manufacturer's protocol, for an additional three days before harvesting culture supernatant for downstream purification processes.

   Note: Optimal harvest time is dependent on the recombinant protein being expressed. We recommend a 6-day fed batch protocol for most secreted proteins.

Legal and Disclaimers

By using this product, the user agrees to the following terms of limited use (https://www.abdesignlabs.com/terms/):

1. Acceptance and Return Policy:

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2. Permitted Use:

This product is intended for research use only. Commercial use is prohibited without a separate license and additional fees. "Commercial use" includes any use of this product or its derivatives for profit or other consideration, such as:

- Product manufacture
- Providing a service, information, or data
- Resale of the product or its derivatives, regardless of whether the resale is for research purposes.

3. Prohibited Uses:

The cell line is not intended for any animal or human therapeutic purposes or direct human in vivo use.

You may not share, modify, transfer, distribute, sell, sublicense, or otherwise make the cell line available to other researchers, laboratories, research institutions, hospitals, universities, or service organizations.

4. Disclaimer of Warranties:

ADL makes no warranties or representations, either expressed or implied, regarding the suitability of the cell line for any particular use.

5. Liability

- ADL accepts no liability in connection with the handling or use of the cell line.

6. Licensing Requirements:

Modifications of the cell line, transfer to a third party, or commercial use may require a separate license and additional fees. Please contact info@abdesignlabs.com for further details.

Related Products

VECTOR	CATALOG	ISOTYPE/USE	DESCRIPTION
Antibody Heavy Chain Expression Vectors			
TGEX™-HC-hG1-Zeo	MX026	Human IgG1	Heavy chain expression plasmid for human IgG1
TGEX™-FH-hG1-Zeo	MX023	Human IgG1 CH1	Expression plasmid for human IgG1 Fab fragments
TGEX™-FC-hG1-Zeo	MX025	Human IgG1 Fc	Expression plasmid for human IgG1 Fc fusions
TGEX™-HC-hG2-Zeo	MX027	Human IgG2	Heavy chain expression plasmid for human IgG2
TGEX™-HC-hG3-Zeo	MX028	Human IgG3	Heavy chain expression plasmid for human IgG3
TGEX™-HC-hG4[S228P]-Zeo	MX029	Human IgG4[S228P]	Heavy chain expression plasmid for human IgG4
TGEX™-HC-mG1-Zeo	MX032	Murine IgG1	Heavy chain expression plasmid for murine IgG1
TGEX™-HC-mG2a-Zeo	MX033	Murine IgG2a	Heavy chain expression plasmid for murine IgG2a
TGEX™-HC-mG2b-Zeo	MX034	Murine IgG2b	Heavy chain expression plasmid for murine IgG2b
TGEX™-HC-mG3-Zeo	MX035	Murine IgG3	Heavy chain expression plasmid for murine IgG3
TGEX™-HC-rbG-Zeo	MX039	Rabbit IgG	Heavy chain expression plasmid for rabbit IgG
Fc-engineered Antibody Heavy Chain Expression Vectors			
TGEX™-HC-hG1[EA]-Zeo	MX041	Human IgG1	IgG1 heavy chain with mutated Fc to increase ADCC/CDC
TGEX™-HC-hG1[NA]-Zeo	MX042	Human IgG1	IgG1 heavy chain with mutated Fc for aglycosylated antibody
TGEX™-HC-hG1[LALA-PG]-Zeo	MX043	Human IgG1	IgG1 heavy chain with mutated Fc to reduce ADCC/CDC
TGEX™-HC-hG1[YTE-KF]-Zeo	MX044	Human IgG1	IgG1 heavy chain with mutated Fc to increase serum half-life
TGEX™-HC-hG4[SPLE-PG]-Zeo	MX045	Human IgG4[S228P]	IgG4 heavy chain with mutated Fc to reduce ADCC/CDC
Antibody Light Chain Expression Vectors			
TGEX™-LC-hK-Zeo	MX030	Human Kappa	Light chain expression plasmid for human Kappa
TGEX™-LC-hL-Zeo	MX031	Human Lambda 2	Light chain expression plasmid for human Lambda 2
TGEX™-LC-mK-Zeo	MX036	Murine Kappa	Light chain expression plasmid for murine Kappa
TGEX™-LC-mL1-Zeo	MX037	Murine Lambda 1	Light chain expression plasmid for murine Lambda 1
TGEX™-LC-mL2-Zeo	MX038	Murine Lambda 2	Light chain expression plasmid for murine Lambda 2
TGEX™-LC-rbKb4	MX040	Rabbit Kappa b4	Light chain expression plasmid for rabbit Kappa b4
Engineering Vectors			
TGEX™-AC- Zeo	MX020	Any expressions	Universal mammalian expression vector
TGEX™-eGFP- Zeo	MX022	Transfection control	Control plasmid for monitoring transient transfections
TGEX™-SCblue-Zeo	MX024	scFv cloning vector	For the transfer of scFv from any pADL phagemid vector and expression as scFv-Fc fusion

Examples for Desired Antibody Format

COMBINATION	FORMAT	PURIFICATION METHOD[1]
TGEX™-HC-hG1-Zeo + TGEX™-LC-hK-Zeo	Full-length human or chimeric IgG1/K	Protein A or G
TGEX™-FH-hG1-Zeo + TGEX™-LC-hK-Zeo	Human or chimeric IgG1/K Fab fragment	Protein L, G or IMAC
TGEX™-FC-hG1	Human IgG1 Fc fusion	Protein A or G
TGEX™-SCblue-Zeo	Human IgG1 scFv-Fc fusion	Protein A or G

[1]Purification by Protein A, G, and L may require additional testing.

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