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Protocol

Protein production in MEXi-293E cells

Preparation and transient transfection of mammalian cells in a synchronized expression system

1 DESCRIPTION

Mammalian cells are often used for the expression of recombinant proteins when complex glycolysation and accurate protein folding are important. The MEXi system provides an affordable mammalian protein expression system along with all essential components. MEXi-293E cells are human embryonic kidney (HEK) cells derived from the 293 cell line, adapted to suspension growth in an affordable cell culturing medium with a low biotin content. This allows highly efficient and specific purification of recombinant proteins via Strep-Tactin®XT under physiological conditions.

The MEXi system uses transient expression rather than stable transformation, to enable a quick and short-term production of the recombinant protein for several days after DNA transfection. This happens via episomal replication and expression of the GOI without the need for a prior integration by making use of the EBNA1 protein in combination with oriP-harboring plasmids. Further, the system deploys a polyethylenimine-based transfection, which is an affordable and quick method suitable even for hard-to-transfect cell lines, while simultaneously avoiding cytotoxic effects as in lipid-based techniques, or complicated viral packaging.

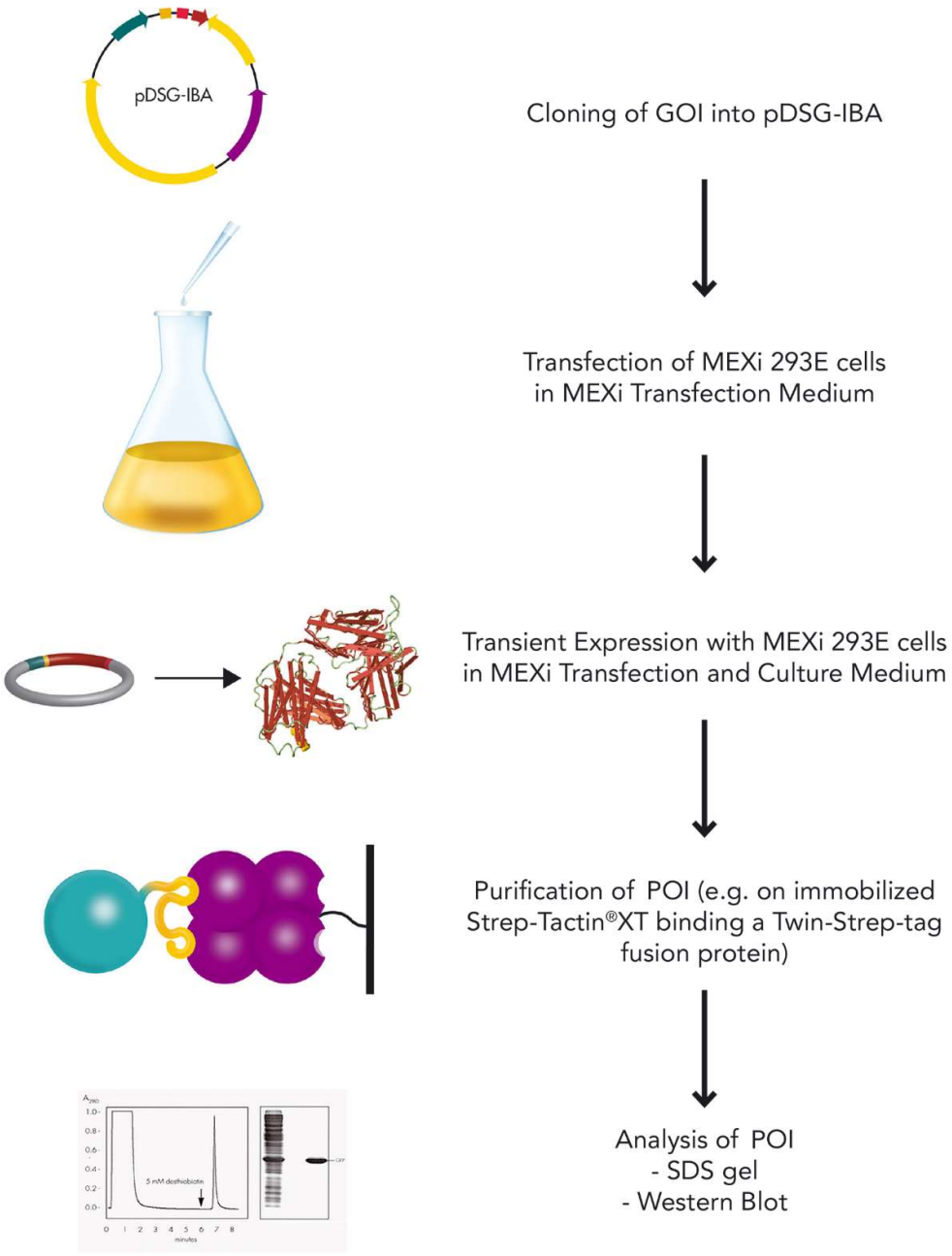
Products beyond protein expression and purification are available from IBA Lifesciences, such as reagents for specific detection, using the Strep-tag® technology. This allows the entire research task to be easily performed using synchronized protocols within one technology platform.

2 GENERAL INFORMATION AND REQUIRED MATERIAL

The following protocol describes the preparation, transfection and expression of recombinant proteins using the MEXi system. The MEXi 293E cell line, a variety of pDSG-IBA expression vectors, MEXi-CM culture medium and MEXi-TM transfection medium are all available from IBA Lifesciences. For optimal results, MEXi 293E cells will have to be thawed from liquid nitrogen, propagated in the optimized MEXi-CM and transferred to the MEXi-TM for transfection. Suitable vectors for transient protein expression driven by the strong, constitutively active CMV promoter are available from IBA Lifesciences, e.g., pDSG-IBA vectors.

Separate protocols for cloning the gene of interest into pDSG-IBA vectors, purification and analysis using products provided by IBA Lifesciences are available at https://www.iba-lifesciences.com/resources/download-area/.

2.1 Workflow


pDSG-IBA
Cloning of GOI into pDSG-IBA
Transfection of MEXi 293E cells
in MEXi Transfection Medium
Transient Expression with MEXi 293E cells
in MEXi Transfection and Culture Medium
Purification of POI (e.g. on immobilized
Strep-Tactin®XT binding a Twin-Strep-tag
fusion protein)
Analysis of POI
- SDS gel
- Western Blot
5 mM desthiobiotin
minutes

2.2 Required materials

2.2.1 Products available from IBA Lifesciences

Reagent	Cat. No.
pDSG-IBA Acceptor Vectors	5-52XX-001
MEXi-293E cell line	2-6001-001
MEXi Culture Medium (MEXi-CM)	2-6010-010
MEXi Transfection Medium (MEXi-TM)	2-6011-010
Products for preparation of protein purification	
Strep-Tactin/Strep-Tactin®XT wash buffer (10x Buffer W)	2-1003-100
BioLock	2-0205-050

2.2.2 Recommended products from other suppliers

Reagent	Supplier	Cat. No.
Distilled water e.g., Ampuwa	Fresenius Kabi	
Dulbecco's PBS (1x)	Capricorn	PBS-1A
G-418 Sulphate (50 mg/ml)	Roth	CP11.3
GlutaMAX (100x)	Gibco	35050
25 kDa linear Polyethylenimines (PEI)	Polysciences	23966
Plasmid DNA MiniPrep Kit	Qiagen	27104
Plasmid DNA MaxiPrep Kit	Qiagen	12162

3 PROTOCOL

3.1 Preparatory steps

Prior to working with MEXi-293E cells, MEXi-CM and MEXi-TM have to be prepared. Add 1.05 ml G-418 sulphate (stock solution: 50 mg/ml) and 42 ml GlutaMAX (stock solution: 200 mM) to 1 l of MEXi-CM or MEXi-TM media resulting in 50 mg/l G-418 and 8 mM GlutaMAX. Store the ready-to-use medium at 4 °C.

3.2 Seed and recovery of cryopreserved MEXi-293E cells

3.2.1. Fill 20 ml of the prepared MEXi-CM into a 125 ml Erlenmeyer flask and place the flask for 60 min in an incubator at 37 °C and 5% CO_2 using gentle shaking to pre-warm the medium and to stabilize pH.

3.2.2 Take the MEXi 293E vial out of the cryo container and quickly transfer it to a water bath at 37 °C for thawing. Clean the outside of the vial with 70% ethanol just before it thaws completely and place it under a clean bench.

3.2.3 Transfer the cells into a 50 ml centrifugation tube pre-filled with 20 ml of 4 °C cold MEXi-CM. Avoid any air bubbles during this step, for example during pipetting, as they introduce shear forces and damage the cells.

3.2.4 Centrifuge the tube for 5 min at 100 x g, 4 °C and discard the supernatant to remove the DMSO from the storage medium (in which MEXi-293E cells are shipped). Re-suspend the cells with pre-warmed medium of step 3.2.1. Transfer the cells into the 125 ml Erlenmeyer flask.

3.2.5 Take an aliquot to determine cell density and viability and incubate the cells at 37 °C and 5% CO_2 on an orbital shaker platform at 110-125 rpm (orbital diameter 50 mm). Subculture cells a minimum of three additional passages after thawing before use in transfection experiments to allow recovery from thawing

3.3 Cultivation of MEXi 293E cells

3.3.1 Subculture cells every 3 to 4 days when cell density reaches 1.5-3.0 x 10^6 cells/ml by diluting into fresh MEXi-CM. Viability should be > 90%. MEXi-293E cells can grow to higher densities without loss of viability. However, in some cases cells show lower transfection efficiency when reaching significant higher cell densities than 3.0 x 10^6 cells/ml during cultivation a few days before transfection.

Determine cell density and viability using standard methods (e.g., Neubauer chamber, cell counter or flow cytometer, etc.).

3.3.2 Seed cells in pre-warmed MEXi-CM (37 °C) at a density between 3.0 x 10^5 cells/ml and 5.0 x 10^5 cells/ml. Observe the maximum filling volumes as outlined in the table below.

Volume shaker flask	Maximum filling volume
125 ml	30 ml
500 ml	150 ml
2000 ml	500 ml

3.3.3 Incubate in humidified air at 37 °C and 5% CO_2 on an orbital shaker platform at 125 rpm (orbital diameter 50 mm).

3.4 Transient transfection of MEXi-293E cells with PEI

Prior to transfection, MEXi-293E cells need to be transferred from the MEXi Culture Medium into the MEXi Transfection Medium. For efficient transfection cells should be seeded at 1.5 x 10^6 cells/ml.

3.4.1 Determine cell density and viability. Viability should be above 90%. Cells with low viability cannot be well transfected. If cell density is above 3.0 x 10^6 cells/ml, transfection efficiency might be reduced.

3.4.2 Pre-warm MEXi-TM in a culture flask at 37 °C and 5 % CO_2 for 1 h to equilibrate pH in the medium.

Thaw the PEI stock solution (1 mg/ml) at 37 °C until the solution is clear. Once the PEI solution is thawed, vortex vigorously for a few seconds.

3.4.3 Allow the DNA solution to adjust to room temperature. When determining the concentration of plasmid DNA in a photometer also check the A260:A280 ratio. This ratio should be above 1.8 to ensure sufficient DNA purity for transfection.

3.4.4 Determine the amount of cell suspension, which is necessary to inoculate the culture flask with 1.5 x 10^6 cells/ml and centrifuge the appropriate amount of cells at 100 x g for 5 min at room temperature (23 °C). Speed and time of centrifugation might be increased (e.g., 300 x g or 10 min) if the supernatant is still turbid after centrifugation.

3.4.5 Discard the supernatant and re-suspend the cells in the appropriate volume of MEXi-TM prepared according to step 3.4.2. We recommend checking the cells under the microscope since best transfection efficiencies can be obtained when the suspension consists of non-agglomerated single cells. This can be achieved through pipetting up and down the cell suspension. Be careful not to overdo it! If small cell agglomerates remain, transfection efficiency will not be affected. Do not vortex the cells. Avoid high sheer forces as it can stress cells and reduce the transfection efficiency.

3.4.6 Inoculate the prepared culture flask (see step 0) to a cell density of 1.5 x 10^6 cells/ml. Add plasmid DNA to a final concentration of 1.5 mg/l directly to the cells and incubate the culture for 10 min on a shaker to achieve a homogeneous suspension.

3.4.7 Add linear PEI drop by drop to a final concentration of 4.5-5.5 mg/l. Shake the flask for a few seconds immediately after adding PEI. A titration (e.g., 4.5, 5.0 and 5.5 mg/l) to determine the PEI concentration providing the most efficient transfection might be necessary, since different PEI formulations from different suppliers as well as different protocols for solving PEI exist. This can lead to different optimal PEI concentrations for the transfection than the concentration provided in this manual.

3.4.8 Shake for 2-4 hours in humidified air at 37 °C and 5% CO_2 on an orbital shaker platform at 125 rpm (orbital diameter 50 mm).

3.4.9 Dilute transfected cell culture 1:2 with MEXi-CM (resulting cell density = 0.75 x 10^6 cells/ml) and continue culturing. A temperature shift to 32 °C shall not be performed prior to cell density has reached 3.0 x 10^6 cells/ml (usually achieved 48 hours post transfection).

3.4.10 Check cell viability regularly. Continue cell culturing until viability drops to 75%. Because MEXi-293E cells can grow to high densities, large cell clumps may form after a few days. Refer to the protocol in Appendix 5.2 to break up cell clumps to increase the accuracy of your measurements.

3.5 Preparation of cell culture supernatant for (Twin-)Strep®-tag affinity purification

We strongly recommend the use of the Twin-Strep-tag® and Strep-Tactin®XT when the recombinant protein is purified from large volumes.

3.3.1 When viability of the transfected culture drops to 75%, harvest cell culture supernatant. Centrifuge cell suspension at 300 x g for 10 min at 4 °C to harvest cells in case of cytosolic expression or to remove cells from the cell culture supernatant in case of secreted proteins.

3.3.2 Add 0.11 volumes 10x Buffer W (e.g., for 1000 ml culture 110 ml 10x Buffer W) to the supernatant. Add 1 ml BioLock Biotin Blocking solution to 1 l of supernatant.

3.3.3 Incubate for 20 min. Long incubation (e.g., overnight) is possible but will not increase purification efficiency.

3.3.4 Centrifuge the supernatant at 10,000 x g for 20 min at 4 C to clear the supernatant and to remove small particles which may clog the column.

3.3.5 Check pH and adjust it, if necessary. Take a sample of the supernatant for analysis. Proceed with purification protocol. For purification Twin-Strep-tag® or Strep-tag®II proteins with Strep-Tactin® the pH should be > pH 7 better pH 8. However, for Strep-Tactin®XT 4Flow® the pH can vary from 4-10.

4 TROUBLESHOOTING

Sequence error, mutation	Verify sequence and reading frame.
Low transfection efficiency	Make sure that the cells are transferred in MEXi Transfection Medium. Optimize DNA and PEI concentration (recommended: 1.5 mg/l DNA; 4.5–5.5 mg/l PEI).
Protein is toxic	Some proteins are inhibiting cell growth or induce apoptosis. In some cases, signaling-inactive forms can be expressed at high levels.
Cell viability (fitness)	If cells are in a high passage or cell growth or viability is low transfection might be inefficient. Use cells which are in logarithmic growth phase (below 3 x 10^6 cell/ml) and show a high viability (e.g., > 90%).
Media conditions	For expression use MEXi-TM and MEXi-CM in a 1:1 ratio.
Protein is not secreted	Check if the signal sequence is present.

5 APPENDIX

5.1 Recommended Cryopreservation of MEXi-293E cells

5.1.1 Bring cells to exponential growth (below 3.0 x 10^6 cells/ml). Cell viability should be higher than 90%.

5.1.2 Prepare cryovials and place them on ice until use.

5.1.3 Immediately before use, mix 900 µl ml of MEXi-CM with 100 µl of DMSO for every ml freezing medium.

5.1.4 Filter-sterilize the freezing medium and pre-refrigerate it at 2-8 °C or place it on ice.

5.1.5 If using “Mr. Frosty” for the freezing procedure, pre-cool it at 2-8 °C.

5.1.6 Determine the cell density. Calculate the volume of cell suspension which is necessary to freeze 1.1 x 10^7 cells per cryovial.

5.1.7 Mix the culture by shaking and pipette the calculated amount of cell suspension into a 15 ml or 50 ml centrifugation tube with conical bottom.

5.1.8 Centrifuge at 100 x g for 5 min at room temperature. Discard the supernatant. The cell pellet must remain in the tube.

5.1.9 Re-suspend cells in the freezing medium to achieve a cell density of 7.33 x 10^6 cells/ml and add 1.5 ml of the suspension into a 2 ml cryovial. **When preparing several vials, place filled cryovials on ice until you transfer them to the refrigerator.**

5.1.10 Transfer the vials into “Mr. Frosty” and place it in a -80 °C freezer for 24 h.

5.1.11 Remove the vials from -80 °C freezer and transfer them into a cryo storage system at -140 °C to -196 °C (in liquid nitrogen).

5.2 Preparation of cells for measuring viability and cell density

During expression of the GOI MEXi-293E cells can grow to high cell densities and form big cell clumps. These cell clumps affect analysis like measurement of cell density and viability. Use the following procedure to break up cell clumps.

If cells do not grow to very high densities (< 4 x 10^6 cells/ml), vigorous vortexing (20-70 seconds) might be sufficient to break up cell clumps.

5.2.1 Place the cell culture flask under a clean bench. Transfer 200 µl of cell suspension into a 1.5 ml reaction tube and centrifuge at 100 x g for 3 min at room temperature. Carefully remove 180 µl of supernatant without disturbing the pellet and discard the supernatant.

5.2.2 Add 180 µl of Dulbecco’s PBS with 5 mM EDTA and 1% BSA, re-suspend the pellet and centrifuge at 100 x g for 3 min at room temperature. Carefully remove 180 µl of supernatant and discard it.

5.2.3 Add 180 µl of TrypLE™ (Gibco, Cat. No. 12604013), re-suspend the pellet and incubate for 2 min at room temperature.

5.2.4 Mix the sample and centrifuge at 100 x g for 1 min 30 s at room temperature. Carefully remove 180 µl of supernatant and discard it.

5.2.5 Add 180 µl Dulbecco’s PBS with 5 mM EDTA and 1% BSA and re-suspend the pellet by pipetting several times.

5.2.6 Vortex the sample vigorously for 30-90 s and proceed with your measurement.

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https://www.iba-lifesciences.com/downloadcenter/

for the latest version of this manual.

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If you have any questions, please contact

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We are here to help!