GenEZ™ ORF clone

User manual

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General information

GenEZ ORF clones contain sequence-verified, protein coding sequences from the NCBI database in expression–ready constructs. GenEZ ORF clones are constructed using the pcDNA3.1⁺/C-(K)-DYK vector. The schematic map for this vector is in Figure 1A. Using CloneEZ® Seamless cloning technology, target ORF is cloned into pcDNA3.1⁺/C-(K)-DYK vector without introducing any extra nucleotides before or after target ORF. This cloning strategy helps to achieve a high expression level and reduce the unknown impact introduced by extra amino acid. The final structure of the GenEZ ORF clone is shown as Figure 1B.

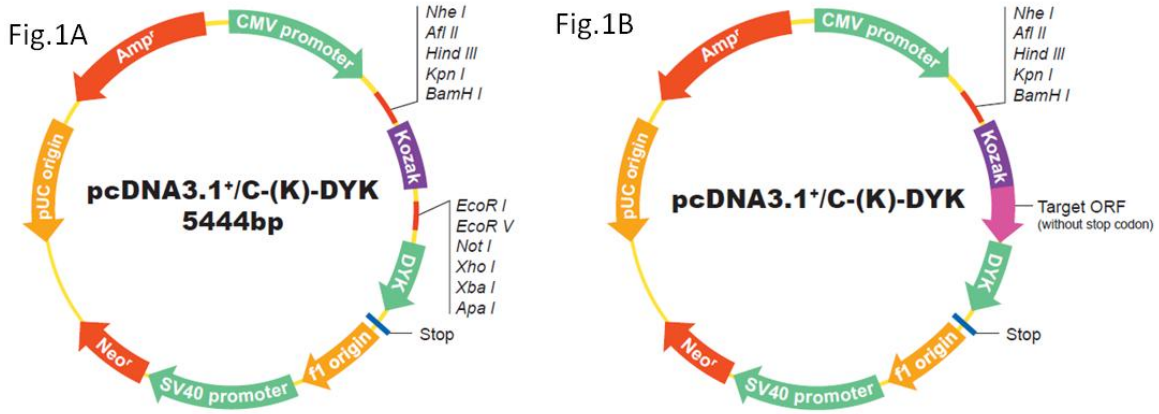

Figure 1. pcDNA3.1⁺/C-(K)DYK vector map (with or without target ORF).

ORFs cloned in pcDNA3.1+/C-(K)DYK vector will be expressed in mammalian cells as a tagged protein with a C-terminal DYKDDDDK tag (DYKDDDDK is the same as FLAG® which is a registered trademark of Sigma Aldrich). Proteins expressed from GenEZ ORF clones can be detected and/or purified following transgene expression using anti- DYKDDDDK antibodies.

User instructions

The GenEZ ORF clone is delivered as 10 µg of lyophilized plasmid DNA in a vial. Keep the vial sealed and store at -20°C for long-term storage.

Before use, centrifuge the vial at 6,000 g x g for 1 minute at 4°C. Open the lid and add 100 µl (or other volume depending on your desired final concentration) of distilled water (or TE buffer) to dissolve the DNA. If necessary, heat the solution at 50°C for 15 minutes to dissolve the DNA. Close the lid and vortex the vial for 1 minute.

Aliquot the dissolved plasmid DNA and store in small aliquots at -20° C.

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Experimental protocol

Protocol for Transfection

A sample protocol is listed here for transfection experiments performed in 6-well plates. To perform transfection experiments in other cell culture plates, simply multiply the suggested quantities by the relative surface area of your plate (see Table 1). Table 1 lists the recommended number of cells to seed per culture plate/dish the day before transfection and the volume of medium to use the day of transfection. GenScript recommends using Lipofectamine® 2000 for all transfections. It consistently produces high transfection efficiency and high protein overexpression.

1. **Adherent cells**: One day before transfection, plate 0.25-1 x 10^6 cells in 2 ml of growth medium without antibiotics per well so that they will be 90-95% confluent at the time of transfection.

Suspension cells: On the day of transfection just prior to preparing complexes, plate 1.0–3.5 x10^6 cells in 2 ml of growth medium without antibiotics per well.

2. **For each transfection sample**, prepare DNA-Lipofectamine® 2000 complexes as follows:

a. Dilute DNA in 250 µl of Opti-MEM® I Reduced Serum Medium without serum (or other medium without serum). Mix gently.

b. Mix Lipofectamine® 2000 gently before use, then dilute the appropriate amount in 250 µl of Opti-MEM® I Medium (or other medium without serum). Mix gently and incubate for 5 minutes at room temperature.

c. After 5 minutes incubation, combine the diluted DNA with the diluted Lipofectamine® 2000 (total volume is 500 µl). Mix gently and incubate for 20 minutes at room temperature to allow the DNA-Lipofectamine® 2000 complexes to form.

3. Add the 500 µl of DNA-Lipofectamine® 2000 complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.

4. Incubate the cells at 37°C in a CO_2 incubator for 24-72 hours until they are ready to assay for transgene expression. It is not necessary to remove the complexes or change the medium; however, growth medium may be replaced after 4-6 hours without loss of transfection activity.

5. **For stable cell lines:** Passage the cells at a 1:10 or higher dilution into fresh growth medium 24 hours after transfection. Add selective medium the following day.

Table 1. Recommended number of cells per culture vessel for transfection.

Culture format	Surface area per well (cm^2)	Adherent cells to seed (day before transfection)	Suspension cells to seed (day before transfection)	Volume of medium
96-well plate	0.3	1.0–4.0 $x10^4$	0.5–2.0 $x10^5$	100 µl
24-well plate	2	0.5–2.0 $x10^5$	2.0–7.0$x10^5$	500 µl
12-well plate	4	1.0–4.0 $x10^5$	0.5–1.5 $x10^6$	1 ml
6-well plate	10	0.25–1.0 $x10^6$	1.0–3.5 $x10^6$	2 ml
60 mm dish	20	0.65–2.5 $x10^6$	2.5–7.5 $x10^6$	5 ml
100 mm dish	60	0.2-7.5 $x10^6$	0.5–2.0 $x10^7$	15 ml

To transfect cells in different tissue culture formats, vary the amounts of Lipofectamine® 2000, DNA, cells, and medium used in proportion to the difference in surface area (see table 1). To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying DNA and Lipofectamine® 2000 concentration, and cell density. Starting points for optimizing transfection in other formats are listed in Table 2. Optimal transfection conditions should be determined for every cell line if the highest transfection efficiency with Lipofectamine® 2000 is required.

Table 2. Recommended starting transfection conditions for Lipofectamine® 2000

Tissue Culture Vessel	DNA (µg) and Dilution Volume (µl)	Lipofectamine® 2000 (µg) and Dilution Volume (µl)	Ratio of DNA:Lipofectamine® 2000
96-well plate	0.2 µg in 25µl	0.5 µg in 25µl	1:0.5 ~ 1:5
24-well plate	0.8 µg in 50µl	2.0 µg in 50µl	1:0.5 ~ 1:5
12-well plate	1.6 µg in 100µl	4.0 µg in 100µl	1:0.5 ~ 1:5
6-well plate	4.0 µg in 250µl	10 µg in 250µl	1:0.5 ~ 1:5
60 mm plate	8.0 µg in 500µl	20 µg in 500µl	1:0.5 ~ 1:5
100 mm plate	24 µg in 1.5ml	60 µg in 1.5ml	1:0.5 ~ 1:5

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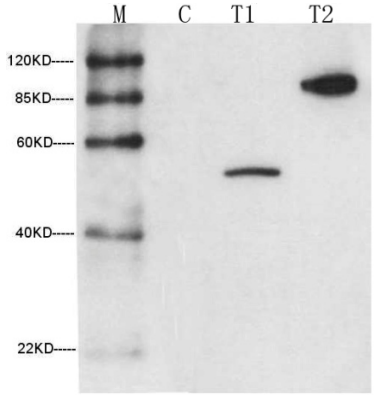
Detection of overexpressed protein using THE™ DYKDDDDK Tag Antibody

The protein expression level can be detected using THE™ DYKDDDDK Tag Antibody (GenScript product number A00187, THE™ DYKDDDDK Tag Antibody) with western blotting method. When GenScript's THE™ DYKDDDDK Tag antibody is used, the suggested protein working concentration is 0.1-1.0 μg/ml for western blot, or 1 μg/ml for immunofluorescent staining and flow cytometry.

Working concentrations for specific applications should be determined by the investigator. The appropriate concentrations may be affected by secondary antibody affinity, antigen concentration, the sensitivity of the method of detection, temperature, the length of the incubations, and other factors.

The lysates used in the western blot below are from the lysates of control HEK293-6E cell or DYKDDDDK-tagged ORF clones.



M: Protein marker (Genscript Cat# MM0908)

C: Control HEK293-6E lysate

T1: Over-expression lysate with IDO1 ORF clone (OHu20074D); Predicted Protein MW: 45kDa

T2: Over-expression lysate with PRKCA ORF clone (OHu19098D); Predicted Protein MW: 77 kDa

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Troubleshooting

For questions not addressed here, please contact GenScript's Technical Support professionals. You may dial toll-free 1-877-436-727. You may also e-mail your inquiries to support@genscript.com.

1. Low transfection efficiency or reduced cell viability following transfection

Possible cause(s)	Suggested solutions
DNA: transfection reagent ratio sub-optimal for cell line	Perform a dose-response curve using both DNA and transfection reagents to determine the appropriate concentration of DNA. And consult the transfection reagents manual.
Plasmid DNA used in transfection has degraded or is of poor quality	Ensure that the plasmid DNA used for transfection is of high quality. Verify concentration or check the DNA for degradation. If necessary, prepare the high quality plasmid DNA again with plasmid preparation kit.
Cell density was not optimal	Normally, efficiency is increased when cell density is between 70% and 90% confluent at the time of transfection.
Plasmid DNA or transfection reagent diluted in media containing serum or complexes formed in the presence of serum	Try serum-free medium for dilutions of plasmid DNA and transfection reagents. Perform a dose-response curve using both DNA and transfection reagents to determine the appropriate concentration of DNA.
Complexes not thoroughly mixed in growth medium	Following addition of transfection complexes into medium, ensure that the plate or wells are thoroughly mixed to prevent concentration of DNA:transfection reagent complexes in the wells
Inhibitors were present in medium	For most of transfection regents, the transfection medium should not contain any antibiotics, phosphates, EDTA, hyaluronic acid, or other sulfated proteoglycans.
Cells changed in culture, or splitting conditions have changed	If transfection performance suddenly declines, it may be because of the cells. Excessive passaging also decreases transfection performance. In this case, obtain fresh cells or new cell line.
Transfection reagent stored improperly	We recommend storing transfection reagents at 4°C. Freezing of transfection reagents, or storing them at room temperature, may decrease activity.
Selection antibiotic added too soon	When creating stable cell lines, allow at least 72 hr for cells to express the resistance gene before adding selective antibiotic.
Problems with assay used to measure efficiency or expression	Use a reporter gene to measure transfection efficiency. A reporter gene control allows you to confirm expression.

2. Transfection results not reproducible

Possible cause(s)	Suggested solutions
Cells have changed over time, or splitting conditions have changed	If transfection performance suddenly declines, it may be because of the cells. Excessive passaging also decreases transfection performance. In this case, obtain fresh cells or new cell line.
Transfections performed at different cell density, or at different DNA: transfection reagent ratios	Transfection performance reproducibility is dependent on day-to-day consistency in cell splitting, plating and transfecting with a consistent protocol (fix the DNA: transfection reagent ratios). Different DNA preparations or media changes may also change transfection performance.

Frequently asked questions

How should I save ORF clones?

Answer: ORF clones should be stored in accordance with the instruction conditions provided upon receipt of the ORF clone. If ORF clones are not preserved in accordance with the instructions, we cannot guarantee the integrity of the DNA.

How should I cite GenScript ORF clones in an article or publication?

Answer: We recommend that you use the product number (unique clone ID) and cite GenScript as the product manufacturer. If your article is published, and you cite GenScript, we will give you a gift of appreciation.

Has GenScript fully sequenced all ORF clones?

Answer: Yes. Each of GenScript's ORF clones are fully sequenced from the 5' end to the 3' end of the ORF insert.

Do ORF clones exactly match the reference gene sequence?

Answer: All ORF clones are guaranteed to match the corresponding ORF sequence posted on our website. However, some clones may contain nucleotide changes compared to the published reference sequences. This is due to SNPs (single nucleotide polymorphisms) reflecting the unique differences from genes expressed in different tissues and different individuals. Published references may represent a different SNP than the GenScript transcript.

What are the sequences of the sequencing primers?

Answer: Forward primer: 5'-TAATACGACTCACTATAGGG-3' Tm=64°C
Reverse primer: 5'-TAGAAGGCACAGTCGAGG-3' Tm= 56°C

Can I transfer large ORFs using this system?

Answer: It has been reported that ORFs larger than 4 kb are unstable in recombination-based systems; conversely, our restriction digest-based vector system has no real size limitation.

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How many amino acids are present in the linker between my protein and the DYKDDDDK-tag?

Answer: There are no extra amino acids between your protein and the DYKDDDDK-tag. The target protein will be fused directly to the DYKDDDDK-tag.

Which vector serves as the negative control for the DYKDDDDK-fusion clone?

Answer: We recommend using pcDNA3.1$^+$/C-(K)-DYK as the negative control.

I cannot detect any protein expression from the ORF clone in the pcDNA3.1+/C-(K)-DYK vector. What are my options?

Answer: 1) Check your transfection efficiency. We recommend using a plasmid that expresses a fluorescent marker (such as, pcDNA3.1$^+$/C-eGFP). 2) Anti-DYKDDDDK antibodies from other vendors are not as sensitive as GenScript's THE™ DYKDDDDK Tag Antibody (A00187) when directed at the same epitope.

If I want to transfer the interested ORF in to other vector, which restriction sites should I selected for subcloning?

Answer: In GenScript, the ORF clone are constructed using CloneEZ® Seamless cloning technology and no restriction site is needed for subcloning. If you want to procedure the subcloning by yourself, you can amplify of the target ORF by PCR with specified enzyme cutting sites and clone into your desired vector.

What is the ORF Guarantee?

Answer: GenScript warrants that the product will meet the specifications listed. At GenScript's discretion, free replacement of any non-conforming product will be made if GenScript is notified within 30 days of product receipt. If you experience any difficulty with any GenScript product, please contact our Technical Support Staff at 1-877-436-727.

Related products and order information

Plasmid DNA Purification Kits	http://www.genscript.com/plasmid_preparation.html
DNA Ladders	http://www.genscript.com/dna_ladders.html
ExpressPlus™ PAGE Gals	http://www.genscript.com/express_plus_page_gels.html
Protein standards	http://www.genscript.com/protein_markers.html
THE™ DYKDDDDK Tag Antibody	http://www.genscript.com/anti_DYKDDDDK_mab.html
Secondary Antibodies	http://www.genscript.com/secondary_antibodies.html