GenEZ ORF Clone Technical Manual

General information

GenEZ ORF clones contain high-quality, proven gene content from the NCBI database in expression–ready constructs.

The GenEZ ORF clone is constructed using the pcDNA3.1^{+}-DYK vector. The schematic map for this vector is in Figure 1.

ORFs cloned in this vector will be expressed in mammalian cells as a tagged protein with an N-terminal DYKDDDDK tag (DYKDDDDK is the same as FLAG® which is a registered trademark of Sigma Aldrich). Proteins expressed from GenEZ ORF clones are the best for the detection and purification of the transgene using anti- DYKDDDDK antibodies.

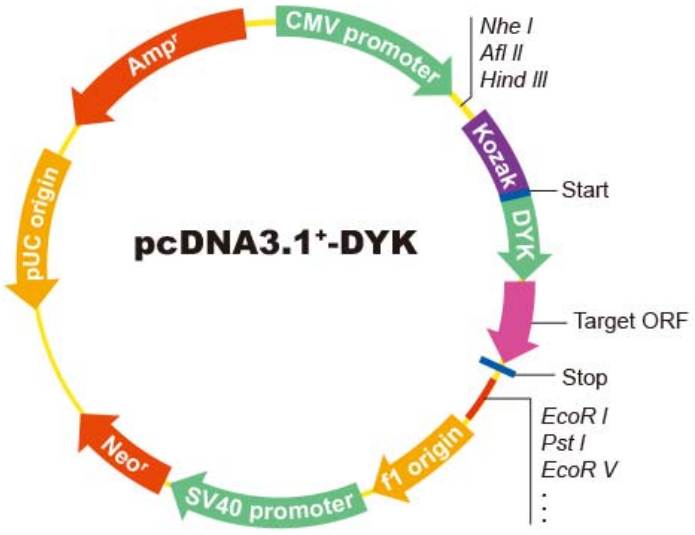


Figure 1. pcDNA3.1^{+}-DYK vector map

User instruction

The GenEZ ORF clone is delivered as 10 µg lyophilized plasmid DNA in vial. Keep the vial sealed and store at 4°C or -20°C for short-term or long-term storage, respectively.

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 and store at -20° C.

Experiment protocol

Protocol for Transfection

A sample protocol is listed here for experiments performed in 6-well plates. To perform 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 ul
24-well plate	2	0.5–2.0 $x10^5$	2.0–7.0$x10^5$	500 ul
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

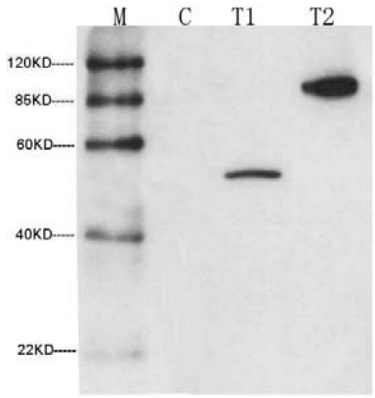
Tissue Culture Vessel	DNA (μl) and Dilution Volume (μl)	Lipofectamine® 2000 (μl) and Dilution Volume (μl)	Ratio of DNA:Lipofectamine® 2000
96-well plate	0.2 μg in 25μl	0.5 μg in 25μl	1:0.5 ~ 1:5
24-well plate	0.8 μg in 50μl	2.0 μg in 50μl	1:0.5 ~ 1:5
12-well plate	1.6 μg in 100μl	4.0 μg in 100μl	1:0.5 ~ 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

Detect protein over-expression 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 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 the control HEK293-6E cell lysate or the DYKDDDDK-tagged ORF clone.



M: Protein marker
C: Control HEK293-6E lysate
T1: Over-expression lysate with ORF-1
T2: Over-expression lysate with ORF-2

Troubleshooting

For questions not addressed here, please contact GenScript's Technical Support professionals. You may dial total free 1-877-436-7274. You may also e-mail your inquiries to support@genscript.com.

1. No colonies or low number of colonies from transformation

Cause	Remedy
The efficiency of competent cells used in transformation was compromised.	Obtain a fresh batch of competent cells and ensure that the efficiency is ≥ 1×10^8CFU/μg DNA by performing a separate transformation reaction with a transformation-qualified control.
The inserted gene is toxic to cells	Growing bacteria at lower temperature, such as 30°C or transforming into strains that reduce the copy number can increase the odds of obtaining colonies.
The transformation efficiency is not high	In some extreme cases, especially for larger inserts (>5 kb), higher efficiency cells or electroporation may be required..

Too little DNA was used in the transformation reaction.	Add more DNA (but not more than 10% of the volume of competent cells used).
The ligation of the ORF donor DNA into the recipient plasmid was not successful.	1) The ligase enzyme may not work properly. Repeat the reaction with fresh ligase and ligation buffer (which contains the temperature-sensitive component, ATP) or perform troubleshooting as recommended by the manufacturer of the ligase. 2) Change amounts and ratios of DNA(ORF insert vs vector) in the reaction.
The antibiotic selection plate was wrong or the antibiotic concentration was too high.	Make sure to use an LB-agar plate containing the correct antibotics (e.g. 100μg/ml ampicillin for destination vectors).

2. Too high self-ligation background (no insert) from destination vector

Cause	Remedy
The destination plasmid was not completely digested.	Allow the digestion reaction to continue for 4 hrs or overnight at 37°C.
The dephosphorylation of the destination plasmid was not complete, and the destination vector religated with its own fragment.	Increase the concentration of antartic phosphatase and/or the length of the dephosphorylation incubation as recommended by the ligase manufacturer.

Frequently asked questions

How should I save ORF clones?

Answer: Customers should store ORF clones 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.

I need to post an article citing your products. How should I write?

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.

How should I cite GenScript ORF clones in an article?

Answer: This can be performed easily using a specific pair of restriction enzymes to cut, ligate, and subclone the ORF into the desired destination vector.

Has GenScript fully sequenced all ORF clones?

Answer: Not always. When transferring the cDNA into the ORF Entry Vector, GenScript always uses fully sequenced plasmids as templates, which have a mutation rate of less than 4 x 10^{-7}. This ensures the highest fidelity of every ORF clone. After cloning into the Entry Vector, each of GenScript's ORF clones are sequenced from both the 5' and 3' ends, and the resulting sequence is matched to the corresponding reference sequence. For ORFs of 1 kb or less in length, the 5' and 3' sequencing reads cover the full ORF. For longer cDNAs, the ORF may not be fully covered by sequencing reads.

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'-CCTCGACTGTGCCTTCTA-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.

What restriction enzymes should I use if Hind III or EcoR I sites are present in my ORF?

Answer: While more than 70% of ORFs can use the HindIII - EcoRI combination, some ORFs do contain internal HindIII or EcoRI site(s). (1) Try to transfer those ORFs using other rare cutters, NheI which is upstream of HindIII; and Not I, whose restriction site is downstream of EcoRI. Using one of the four different subcloning combinations, any ORF can be transferred from one vector to another. The recommended subcloning combination for every ORF cDNA is listed in the product information on our website. (2) Amplify of those ORFs by PCR with specified enzyme cutting sites and clone into your desired vector.

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 pcDNA3.1+.

I cannot detect any protein expression from the ORF clone in a pcDNA3.1+-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^{+}-DYK). 2) Anti- DYKDDDDK antibodies from other vendors are not as sensitive as GenScript's THE^{TM} DYKDDDDK Tag Antibody (A00187) when directed at the same epitope.

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 1-877-436-7274.

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