Pre-assembled cDNA clone sets

Transfection-Ready Clone Sets

Cat# TCTF101, TCTF102

TCPK101, TCPK102

TCGR101, TCGR102

TCSP101, TCSP102

TCTM101, TCTM102

Application Guide

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Package Contents and Storage Conditions

Product Contents

- 90 lyophilized transfection-ready cDNA clones (100 ng per well) arrayed in a 96-well plate
- Certificate of Analysis

Storage Conditions

Shipped at room temperature. For long-term storage, the product should be stored at -20°C sealed. Once the seal is broken, the plate needs to be used immediately.

Notice to Purchaser

- This product is for research use only. Use in and/or for diagnostic or therapeutic purposes is strictly prohibited.
- Reverse engineering of the assay plate including isolation or copying of any individual clone is not allowed.

Other Required Materials and Reagents

- Low toxicity transfection reagent suitable for transfection with serum such as TurboFectin 8 (cat# TF81001)
- Opti-MEM I medium
- Routine tissue culture supplies
- Transfectable cell line (such as HEK293 cells)

Optional Reagents and Equipment

- A GFP vector for transfection positive control and optimization, such as pCMV6-AC-GFP (cat# PS100010).

Related Products

TrueClone™ cDNA clones
Tagged ORF clones
RNAi
Transfection reagents
Antibodies
Recombinant proteins
CRISPR

Introduction

The pre-assembled cDNA clone sets of specific gene families are designed for high throughput functional screening by protein overexpression. The clone sets include transfection factors, protein kinases, GPCR, secreted genes and transmembrane proteins.

The clone sets contain expression-ready cDNA clones arrayed in a 96-well format optimized for high-throughput transfection and rapid screening. Each well contains a standardized amount of plasmid DNA (100ng) from OriGene's large selection of human cDNA full-length clones (TrueClones) in the robust pCMV6 mammalian expression vector. The cDNA clones are tag-free, so native proteins will be expressed. The procedure is simple: just add transfection reagent and cells to the plates, then do the functional assays after 48 hours. Gene over-expression provides additional information over other methods like gene silencing by providing gain of function screening and the ability to target lethal genes that cannot be silenced.

If larger clone sets or custom clone sets are needed, cherry-picking clone sets from gene families and pathways are available online: https://www.origene.com/products/cdna-clones/expression-plasmids/clone-set

An overview of the procedure is presented in the diagram below.

Figure 1. Simplicity of using the pre-assembled clone sets

Optimization of Transfection & Assay Conditions

Before embarking on a broad use of the arrayed clone sets, it is important to optimize your transfection conditions, validate your assay readout, and identify the best conditions to maximize the assay signal to noise ratio. Optimization should include:

- Identifying the most appropriate transfection reagent
- Choosing the most effective DNA to transfection reagent ratio

- Choosing the optimal number of cells to be used
- Validating your reporter gene (when applicable) and assay controls

Selecting your transfection reagent

A low toxicity serum compatible reagent must be used, such as TurboFectin from OriGene (cat# TF81001). TurboFectin is a robust transfection reagent that can efficiently deliver DNA into a wide spectrum of cell lines. For a specific cell line, you may need to find the best transfection reagent. OriGene offers several plasmid DNA transfection reagents that work for different types of cells. For further details, see our website at: https://www.origene.com/products/others/transfection-reagents

Optimization of Transfection

The clone set arrays are designed for high throughput reverse transfection protocol. In reverse transfection, DNA and the transfection reagent are added to the plate first and allowed to form a complex before cells (in suspension) are added. With this method, cells are transfected as they settle down and adhere to the plate. Reverse transfection is easier and faster to perform in high throughput than traditional transfection. Reverse transfection is compatible with most cell types and many transfection reagents. However, transfection conditions should be optimized for your cells and transfection reagent. A fluorescent vector can be used to monitor transfection efficiency, such as pCMV6-AC-GFP vector from OriGene (cat# PS100010). Please optimize the transfection conditions according to the manufacturer's protocol.

Transfection Protocol Using HEK293 Cells and TurboFectin

1. Reconstitute the lyophilized DNA in Opti-MEM media
    1) Add 50 µL of Opti-MEM I to each well to reconstitute the plasmid DNA. If a reporter gene is needed for your assay, the total volume of Opti-MEM and the reporter gene is 50 µL; reconstitute the DNA in the well using Opti-MEM, then add the reporter gene. Remember not to add anything that will interfere with the transfection such as serum or proteins.
    2) Centrifuge briefly to bring all the volume down and shake on a microplate mixer for 10 minutes. This step will ensure obtaining maximal amount of the spotted plasmid DNA.
    3) If negative control or positive control DNA needs to be added to the plate, add 50 µL of the DNA (containing 100 ng) in Opti-MEM.

2. Dilute TurboFectin solution in Opti-MEM media

Prepare your dilution of TurboFectin at 50 µL / well (up to 75 µL can be used) based on your optimization assay. The ratio of TurboFectin to DNA is 3:1; 3 µL of TurboFectin to 1 ug of DNA. For each 100ng DNA well, the amount of TurboFectin is 0.3 µL. Remember

to account for the added amount of the reporter plasmid (if needed) (100 ng spotted plasmid + reporter plasmid).

3. Add 50 µL of the diluted TurboFectin solution to each of the wells.
Centrifuge briefly and shake on a Microplate mixer for 30 seconds.
Incubate at room temperature for 15-30 minutes or according to the suggested manufacturer conditions or your specific cell requirements.

4. Add 100 µL of HEK293 cells (10,000 – 20,000 cells) to each well.
While waiting for the incubation time in step 3, prepare for HEK293 cells, which should be at confluency lower than 75% (for HEK293T cells).
    1) Trypsinize HEK293 cells and re-suspend in complete DMEM.
    2) Count cells and dilute in complete DMEM media at 10^5 cells/mL (10,000 cells in 100 µL). For your specific cells, different amount of cells may be needed due to different cell sizes and your assay requirement.
    3) Add 100 µL of the diluted cells to each of the wells.

5. Cover the plate and incubate at 37°C in a CO_2 incubator for 48 hours. Then do your functional assays.

Note: All procedures related to cells must be carried out in a sterile manner. We recommend using antibiotics in the complete medium, but this may reduce the efficiency of the transfection.

Frequently Asked Questions

Why are there only 90 genes in each clone set?
In order to make the clone set arrays affordable only 90 genes/cDNA clones are included for each set. Two different sets are offered for each gene family. If you need to screen more genes, you can get both sets. If additional genes are needed, custom clone sets can be requested at: https://www.origene.com/products/cdna-clones/expression-plasmids/clone-set .

How much DNA can be recovered from each well?
We are able to recover close to 90% and higher of the added plasmid DNA. Following our procedure and using the recommended microplate mixer are important for maximal recovery of DNA.

Which transfection reagents do you recommend?
We routinely use the TurboFectin 8 transfection reagent. However, other non-toxic transfection reagents may work as well depending on your cell type and assay.

How important it is to optimize the transfection conditions?

We found that changing the ratio of the transfection agent from 3:1 to 3:2 (µL/ug DNA) results in considerable reduction of transfection. Unless you use HEK293T cells or any other cell lines that were optimized by OriGene for reverse transfection it is critical that you optimize the conditions of your transfection reaction. Failing to do so can result in negative outcomes.

Why are clone set arrays constructed by first coating the plates with pUC-19?
OriGene has found that pre-coating the plates with a plasmid like pUC-19 prevents the loss of the target plasmid by adherence to the plate. More importantly, it is well known that transfection efficiencies are higher when a small percentage (10%, w/w) of a second, non-reactive plasmid is included as part of the experiment.

Why can't I use the clones provided in the array for any purpose besides screening?
The TrueClones provided in the array are proprietary products of OriGene available as individual clones. Charging our regular or even a substantially reduced price will make the array outside of the reach of most researchers. To overcome this problem, the clones in the array are provided for screening only. Individual clones to validate your results can be purchased from OriGene and used for any *in vitro* purpose.