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Product Information

CompoZr® Custom Zinc Finger Activators
CompoZr Custom ZFA, Rep, Epigenetic Mod

Catalog Numbers CSTZFACT-1KT
CZFA2136-1KT (ZFA targeting human SOX2)
CZFA17184-1KT (ZFA targeting human OCT4)
CZFA1504-1KT (ZFA targeting human KLF4)
CZFA1041-1KT (ZFA targeting human c-MYC)

Technical Bulletin

Important
This protocol is optimized for use with TransIT LT®-1 (Mirus Bio)
Other suggested transfection reagents include:
TransIT®- 2020 (Mirus Bio)
Lipofectaime® 2000 (Invitrogen)

Any transfection reagent may require additional optimization of the protocol

Product Description

Various classes of naturally occurring DNA-binding molecules, including zinc-finger proteins (1,2), triplex forming oligos (TFOs) (3), meganucleases (4), transcription activator–like effectors (TALEs) (5,6), and the recently discovered *cas9* proteins (7,8), have been engineered to bind sequence-specific endogenous target sites. Such DNA-specific binding proteins, when coupled to various functional domains, are powerful tools for modifying the genome or regulating gene expression. Zinc finger proteins have been highly characterized for such uses (1,2). These modular proteins consist of multiple zinc-finger domains, each recognizing 3 base pairs of DNA linked together to generate a protein that binds specific DNA sequences. When fused to a transcriptional activation domain (such as VP16 from the herpes simplex virus), these engineered transcription factors (ZF-TFs) effectively upregulate target gene expression when delivered to cells (9-14).

The zinc-finger domain for each ZF-TF consists of either five or six zinc-finger subunits, resulting in a ZF-TF with either a 15 or 18 base-pair DNA recognition site. Each 5 or 6 ZF coding sequence assembly is cloned between an N-terminal nuclear localization signal and a C-terminal NF-κB p65 activation domain to generate a series of ZF-TF expression constructs. The resulting fusion protein is designed to bind to the specific target sequence on either the forward for reverse strand of DNA. The ZF-TFs are then screened in HEK293 cells (for human gene targets) or Neuro 2A cells (for murine targets) by transfection with TransIt-LT1 (Mirus Bio). Measurement of target gene mRNA is used to determine whether designed ZF-TFs successfully upregulate the target gene's expression (Figure 1).

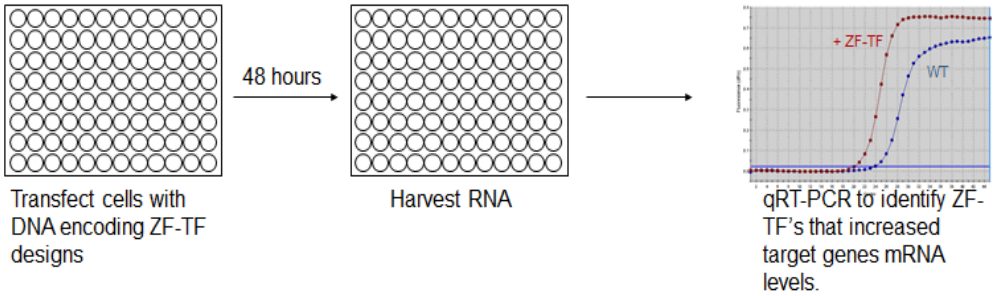

Figure 1. Schematic of the ZF-TF Validation Assay

Cells are harvested 48 hours post-transfection, and total RNA is isolated using RNeasy Plus 96 Kit (Qiagen) or RNeasy Plus Mini Kit (Qiagen). The levels of target gene mRNA and *PPIA* (cyclophilin A) endogenous control (Applied BioSystems) mRNA are measured by qRT-PCR using Quantitative RT-PCR ReadyMix (Sigma-Aldrich Biotechnology), following the manufacturer's protocol. qRT-PCR analysis is performed using the Mx3000P (Stratagene).

Unvalidated ZFAs are also available. For the unvalidated ZFAs, up to 12 ZFA plasmids (with the p65 activation domain) will be shipped. Please note that the qRT-PCR validation assay is not included with the unvalidated ZFA products.

Optimizing ZF-TF activity via effector domain selection

The effector domain linked to a ZF-TF can have a large influence on the level of transcriptional activation, repression, or modification achieved by such artificial transcription factors (16). Using ZF-TFs identified in the screening method above, the end user can further optimize targeted gene activation by fusing different effector domains to the zinc-finger domains in place of the NF-κB p65 activation subunit.

In order to demonstrate the varied efficacy of each domain in the context of individual target gene activation, ZF-TFs targeting *OCT4* (CZFA17184-1KT), *SOX2, (*CZFA2136-1KT), *KLF4* (CZFA1504-1KT*),* and *c-MYC* (CZFA1041-1KT) were tested with p65, VP16, VP64 or 2Xp65 (two tandem copies of the p65 domain) activation domains (Figure 2).

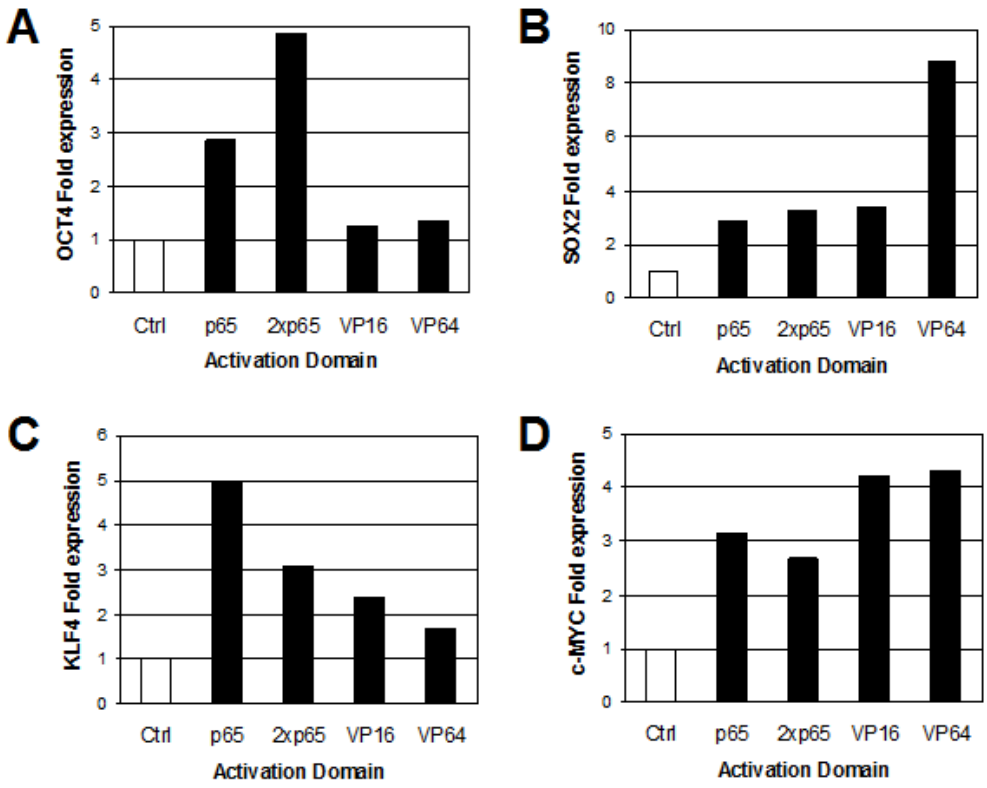

Figure 2. Use of various effector domains with ZFPs to upregulate mRNA expression.

ZF-TF Plasmid Design and Cloning Options

ZF-TF molecules are delivered in plasmid format, in the vector backbone shown below (Figure 3). Expression of ZF-p65 is driven by the CMV promoter, along with an upstream GFP-2A cDNA, allowing for visual selection of cells that contain your plasmid of interest. This in turn allows for more accurate expression data analysis for both in vivo and in vitro experiments.

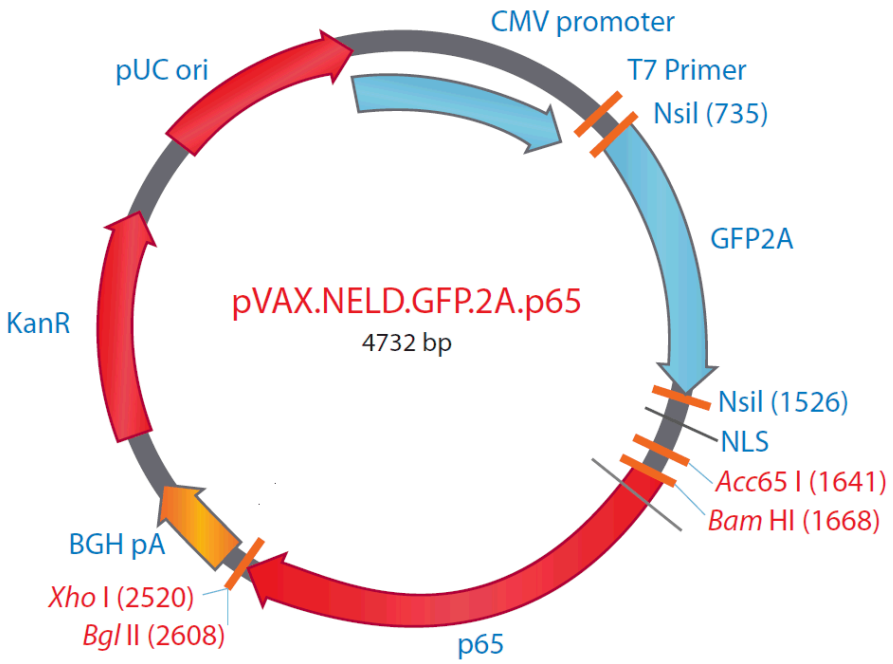

Figure 3. Map of the ZF-TF cloning vector (with p65 effector domain).

Cloning to alternate and remove effector domains

Standard format for ZF-TF delivery is via the plasmid containing the p65 activation domain. Zinc finger motifs are cloned 5' of this sequence using Acc65I and BamHI sites (see Figure 3 for reference). The necessary plasmid backbones that contain additional activation domains (VP16, VP64, or 2xp65), repressor domain (KRAB), or epigenetic modification domains (p300 or G9A) are provided along with the standard p65 plasmid. To manipulate the domain on each ZF-TF, a simple transfer of the zinc finger module to the destination plasmid will be required, using Acc65I and BamHI to perform the restriction digestions. Upon ligation into the destination plasmid, the zinc-finger module will now be in frame with the downstream activation domain, resulting in a ZF-TF fusion protein. In addition, a zinc-finger only control (lacking the transactivating domain) can be created by simple digest of the original ZF-TF-p65 plasmid with BamHI and BglII, followed by purification and intramolecular ligation of the larger DNA fragment containing the zinc-finger sequence. These ends are cohesive and compatible and can be ligated together to form your domain drop out vector, which can be used for control experiments.

General Guidelines for Transfection

Given that the ZF-TF product is delivered in plasmid format, reagents should be immediately ready for small-scale transfection, unless scale up and alternate domain optimization is desired. While this product has been optimized using TransIT LT®-1 (Mirus Bio), use of a variety of transfection methods and reagents is possible. It is expected that the end user will optimize transfection conditions based on cell type of interest and any downstream assay constraints. For best results, Sigma-Aldrich recommends a starting range 0.5 to 1.5 micrograms of ZF-TF plasmid per well in either 24- or 12- well plates, containing 50,000 to 100,000 cells per well. Optimization within this range should generally yield at least a 2-fold change in target gene expression for a validated ZF-TF. It is assumed that these results may vary based on cell type and transfection method of choice.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage and Stability
Recommended storage of DNA is -20C. Bacterial glycerol stocks should be stored at -80C.

RNases are ubiquitous and very stable proteins, which are a primary concern for any researcher attempting to manipulate RNA. Care must be taken not to introduce RNases. It is recommended to use RNase-free pipette tips, preferably those having an aerosol barrier, to wear latex gloves and change them frequently, and keep bottles and tubes closed when not in use.

Kit components
For each custom order, the zinc finger corresponding to the target of choice will be provided in the following format: ZF + C-terminal NF-κB p65. Up to three ZFAs will be provided. Additional cloning vectors are provided according to the table below.

Reagent Description	Catalog Number	CSTZFACT – 1KT	CSTZFACT – 1KT Upgrade
pZFA1	D1ZFA	1 EA	1 EA
pZFA2	D2ZFA	None or 1 EA	None or 1 EA
pZFA3	D3ZFA	None or 1 EA	None or 1 EA
pZFA-GFP-vp16	ZFG0002	1 EA	1 EA
pZFA-GFP-vp64	ZFG0003	1 EA	1 EA
pZFA-GFP-2xp65	ZFG0004	1 EA	1 EA
pZFE-GFP-p300*	ZFG0005	N/A	1 EA
pZFE-GFP-G9A*	ZFG0006	N/A	1 EA
pZFR-GFP-KRAB*	ZFG0007	N/A	1 EA

*The vectors that contain the p300, G9A and KRAB domains are available as an optional upgrade to the custom ZFA kit.

Note: For the unvalidated ZFAs, up to 12 ZFA plasmids (with the p65 activation domain) will be shipped. Please note that the qRT-PCR validation assay is not included with the unvalidated ZFA products.

Reagents and Equipment Recommended But Not Provided

Nucleofection® reagents and instrument or Electroporation reagents and instrument or *Trans*IT®-mRNA Transfection Reagent (Mirus Bio Catalog Number MIR 2225, LLC)
Agarose
Hank's Balanced Salt Solution (HBSS, Catalog Number H6648)
DirectLoad 1 kb DNA Ladder (Catalog Number D3937)
Quantitative RT-PCR Ready Mix (Catalog Number QRO200)

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