

Product Information

COMPOZR® KNOCKOUT ZFN KIT - CHO SLC35A1

CATALOG NO. ZFN_{SLC35A1}

Product Information

Zinc Finger Nucleases (ZFNs) are a class of engineered DNA-binding proteins which facilitate targeted genome editing by binding to a user-specified locus, subsequently causing a double-strand break (DSB). Post cleavage of the DNA, the cell employs endogenous DNA repair processes, either non-homologous end joining (NHEJ) or homology-directed repair (HDR), to repair this targeted DSB. These repair processes can be channeled to generate precisely targeted genomic edits resulting in a cell line or an organism with specific gene disruptions (knockouts), integrations, or modifications. To provide the best potential of creating a functional gene knockout, CompoZr Knockout ZFNs are targeted to the 5' exon sequences which represent the first 2/3 of the coding region for the gene of interest.

Gene Target Background

The protein produced by the SLC35A1 gene resides on the membrane of the Golgi and is responsible for transporting the nucleotide sugar CMP-sialic acid into the Golgi. Knocking out function of this gene in a cell results in the production of asialylated glycan structures due to the loss of available sialic acid as a substrate to downstream sialic acid transferases.

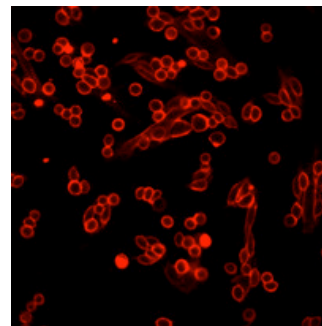
Attachment to a cell surface receptor is a key first step in the infection cycle for many viruses (Figure 3). It has been shown that viral capsids derived from Minute Virus of Mice (MVM) bind to specific glycan structures with a terminal sialic acid. Knocking out the function of this gene in a cell results in asialylated glycan structures (Figure 1), thus eliminating the ability of MVM to bind to and enter the cell (Figure 2). Therefore, a SLC35A1 knockout host cell line can be engineered leveraging the ZFN technology in order to create a MVM resistant cell line.

SAFC has identified a ZFN pair targeting the CHO SLC35A1 gene (Gene ID 100689322). Upon genetic disruption at this loci, asialylated cell lines can be isolated. SAFC has validated this phenotypic affect, as well as resistance to MVM infection, by making SLC35A1 knockouts using the CHOZN GS^{-/-} cell line (Product No. CHO_{GS}).

Kit Components

CompoZr Knockout ZFNs are provided as aliquots (10 per kit) of mRNA encoding for the ZFNs along with plasmid DNA for both ZFN pairs. Also included in the kit are custom-designed PCR primers that allow for determination of the rate of mutation and for screening of individual clones that have the desired mutational event, and a positive control (genomic DNA that has been modified using the CompoZr Knockout ZFN).

Wildtype CHO



SLC35A1 knockout CHO

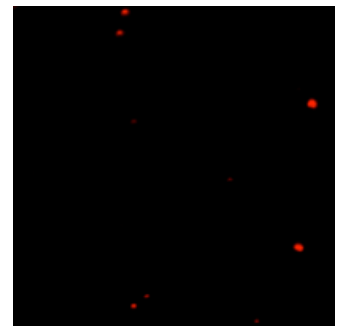


Figure 1. Wildtype and knockout cells were incubated with MAL II-biotin, washed and then incubated with Streptavidin-Alexa-647. Cells were washed and observed under a microscope. SLC35A1 knockout cells do not bind MALII lectin indicating the absence of surface sialic acid.

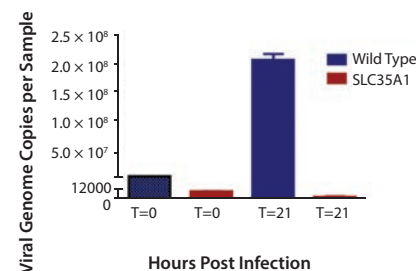
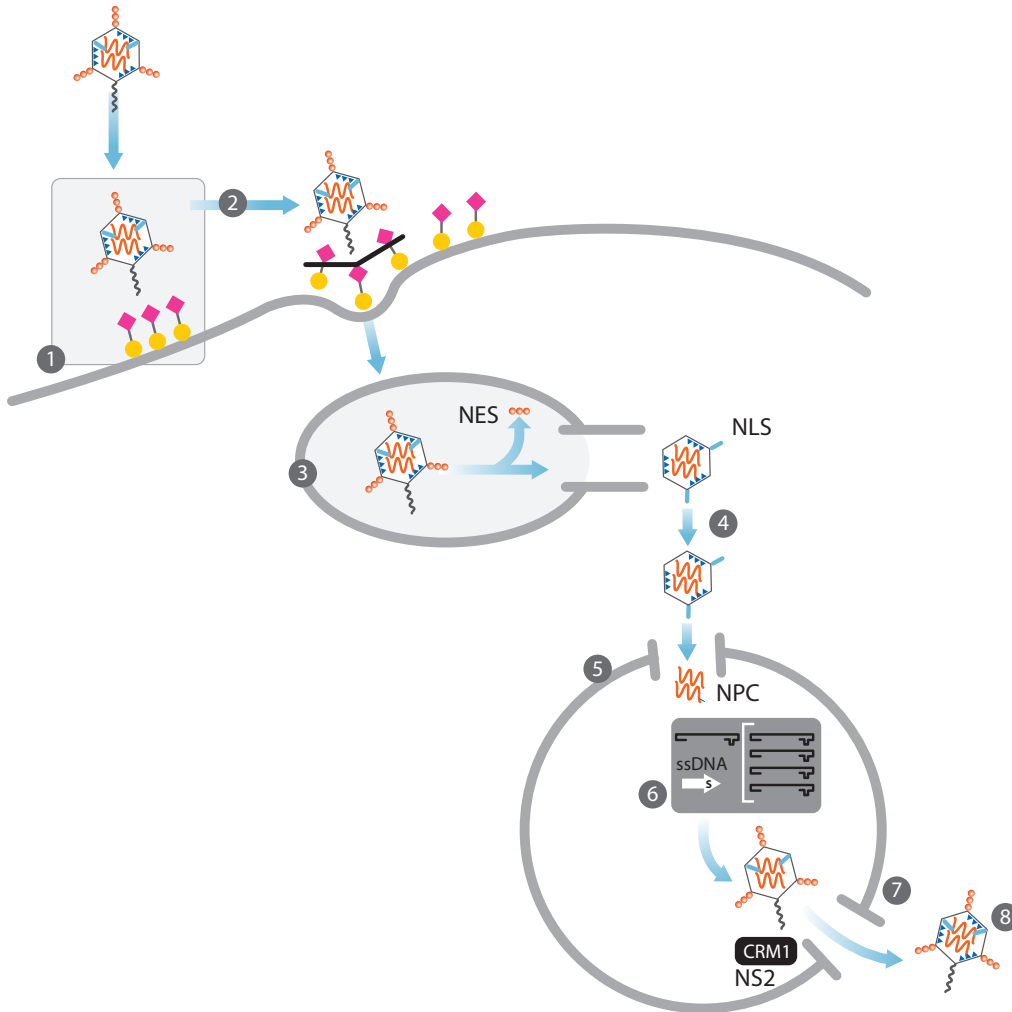


Figure 2. Wildtype and SLC35A1 knockout cells were incubated with MVM for 3 hours and then washed to remove non-specifically bound virus. Samples were then collected at time=0 hours and time=21 hours and analyzed by qPCR using a probe against the MVM NS2 gene. Time 0 indicates levels of viral attachment. Time 21 indicates levels of internalization and single cycle replication.

Features and Benefits

- First commercially available gene editing tool for viral resistance applications
 - Enables the generation of a MVM resistant host CHO cell line
 - Can be used to engineer a host cell line or a producing cell line
- Full kit provided with controls
 - Ten aliquots of transfection ready mRNA
 - Plasmid DNA provided for additional RNA generation or expansion
 - Primers for screening and sequencing
 - Positive control DNA for CEL I assay validation
 - Technical bulletin provided with detailed protocols for transfection and ZFN activity confirmation
- Technical support provided by the CHOZN R&D scientists
- Custom Cell Line Engineering services are available – inquire at CHOZN@sial.com



- 1 Virus bind to sialic acid
- 2 Galectin 3 stabilizes binding or bridges to secondary receptor
- 3 Virus enters in an endosome. exit is inefficient and depends on virion phospholipase
- 4 Vimentin required to transport virion to nuclear membrane
- 5 Entry into nucleus requires breakdown of lamin which is facilitated by Caspase 3
- 6 Replication in nucleus of mitotically active cells
- 7 Exit of viruses is an active process and requires breakdown of actin filaments
- 8 Final processing of progeny virions in Golgi involves phosphorylation of the capsid

— VP1
 ○ VP2
 — 2Nt

Figure 3. Infection and replication of Minute Virus of Mice in a host cell line

By eliminating the presence of surface sialic acid via ZFN mediated knockout of SLC35A1, MVM is unable to infect and replicate within a host cell line.

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