bio-techne® / R&D SYSTEMS

Catalog Number	Mass
TCB-001.1-100	100 µg
TCB-001.1-500	500 µg

TcBuster-M™ Transposase mRNA

Non-Viral Gene Delivery Reagent

Product Description

TcBuster-M transposase mRNA encodes a hyperactive version of TcBuster transposase. TcBuster is a non-viral DNA transposon system that enables gene transfer and stable integration in mammalian cells (Figure 1). TcBuster-M transposase, a member of the hAT superfamily of transposons, was originally isolated from the red flour beetle, Tribolium castaneum, before undergoing directed evolution (1,2). TcBuster mediates transposition of DNA cargo flanked by inverted terminal repeats (ITRs) that facilitate transposase binding.

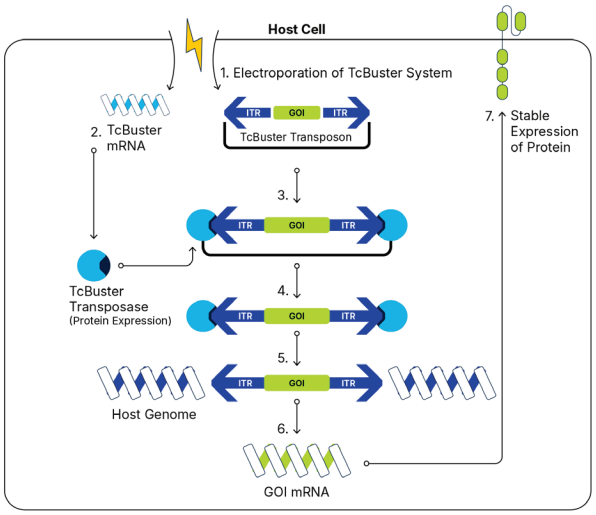
Attribute	Description
Manufacturing Process	*In vitro* transcribed mRNA, including polyadenylation and capping
mRNA Length	2154 nt
Capping Mechanism	M7G
Concentration	≥1.0 mg/mL
Formulation	1mM Sodium Citrate, pH 6.4

Intended Use

The TcBuster system is a versatile genome editing tool and has applications in a wide variety of cell types. Examples include, but are not limited to, the transposition of immune cells (T, NK) (3,4), induced pluripotent stem cells (iPSCs), and bioproduction cell lines (CHO, HEK, etc.). Broad application will require independent experimental optimization and/or process development. If you have any technical questions about the TcBuster DNA transposon system, contact our TcBuster scientific support team at techsupport@bio-techne.com for assistance.

FIGURE // 01

The TcBuster Non-Viral Gene Delivery System



1. TcBuster system reagents are introduced into the cells via electroporation.
2. TcBuster-M mRNA is translated into TcBuster-M transposase enzyme.
3. TcBuster-M transposase binds to inverted terminal repeats (ITRs) on the TcBuster DNA transposon.
4. TcBuster-M transposase cuts gene(s) of interest (GOI) from transposon.
5. TcBuster-M inserts GOI into the host genome.
6. Inserted GOI is transcribed into mRNA.
7. Translated GOI protein is stably expressed in cells.

Storage & Stability

TcBuster™ is an mRNA product and is provided in 1 mM Sodium Citrate, pH 6.4 formulation. Store TcBuster-M™ at ≤ -70 °C. Use a manual defrost freezer and avoid repeated freeze-thaw cycles by making single use aliquots at your desired volume.

Precautions

This product must be handled with the utmost care to prevent product degradation. It is recommended to limit RNAses in the work environment when handling TcBuster-M. TcBuster-M is subject to degradation upon exposure to environmental RNases, especially those found in human saliva, hair, and skin, as well as those originating from target cells. Appropriate PPE must be employed to protect product integrity: face shield or procedure mask, hair net, clean lab coat or gown, Tyvek® sleeves, and clean nitrile gloves. Do not touch containers or tubes with bare hands. Use aseptic technique as appropriate when working with mRNA. Use RNase decontamination agent. Refer to appropriate Safety Data Sheet when working with chemicals.

Limitations

- For laboratory research use only. Not for use in diagnostic procedures.
- For certain applications, results may vary due to differences in donor derived cell populations.

Reagents & Equipment

REQUIRED

- TCBP001: TcBuster Transposon CD19CAR-DHFR-eGFP
- TCBP002: TcBuster Transposon Insert On eGFP
- For custom TcBuster compatible transposons, contact techsupport@bio-techne.com for more information.
- Electroporation platform of user's choice

RECOMMENDED

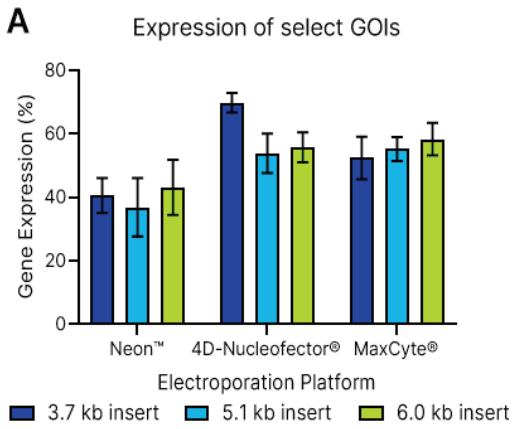
RNase decontaminant and RNase free environment to safeguard against RNase contamination.

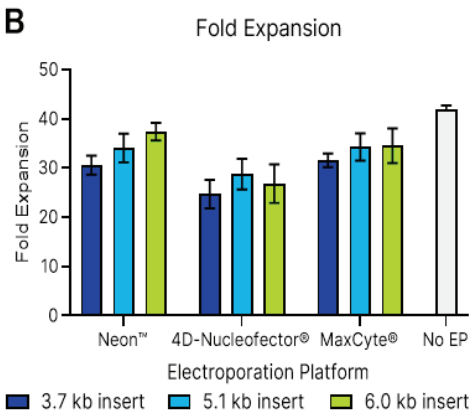
- RNase inhibitor
- RNase decontamination agent
- Proper PPE
- Cell washing

Reagent Preparation

This preparation is suggested to limit RNase exposure. TcBuster-M comes ready to use and should be stored as supplied at ≤ -70 °C. It is recommended to thaw TcBuster-M on ice for 30 minutes or until thawed and aliquot into smaller, single use working aliquots, which are then stored at ≤ -70 °C.

Data Examples





TcBuster-M transposes a range of TcBuster transposons using multiple electroporation platforms and maintains robust fold expansion. Pre-activated T cells were transposed using 1 µg TcBuster-M + 10 x 10^6 cells + TcBuster specific transposons. T cells were electroporated using either the Neon™, 4D-Nucleofector®, or the MaxCyte®. Edited T cells were plated in a 6-well G-Rex® and grown out for 7 days post-electroporation. (A) Transposition efficiency analyzed by flow cytometry of 3 different sized genes of interest (GOIs) 9 days post- T cell thaw and activation. No EP not shown, as no transgene expression was detected. (B) Fold expansion of heterogenous T cell grow outs 9 days post T cell thaw and activation.

Product Specific Notices

Transfer of TcBuster Transposase (”Product”) is strictly prohibited. The Product is to be used for research use only, and not for clinical or commercial purposes. The Product is the wholly owned intellectual property of Bio-Techne and unauthorized parties do not have the right to make or have made the Product, or any portion thereof. Full details of R&D Systems™' Terms and Conditions of sale can be found online at: https://www.bio-techne.com/terms-and-conditions.

REFERENCES

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2. 2. Woodard, L.E et al. (2012) PLOS One DOI: 10.1371/journal.pone.0042666
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4. 4. Gurney, M et al. (2022) Cytotherapy DOI: 10.1016/j.jcyt.2022.07.008