PEmax Enzyme

Manual

Catalog #	3473	3476
Package Size	80 µg	400 µg

Important!

-20°C Storage Required

* Immediately inspect packages
* Freeze upon receipt

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Intact Genomics, Inc.

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Description:

CRISPR-associated (Cas) systems

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids[1]. The CRISPR system consists of a short noncoding guide RNA (sgRNA) made up of a target complementary CRISPR RNA (crRNA) and an auxiliary transactivating crRNA (tracrRNA)[2]. Prime-editing is gaining popularity as a precision gene-editing technique. Prime-editing enzymes are a fusion of the Cas9 nickase mutant (H840A) with a modified Murine Moloney Leukemia Virus-Reverse Transcriptase (MMLV-RT). One of the resulting optimized prime-editing fusions is known as PEmax enzyme[3-4]. This prime-editing enzyme has enabled precise gene-editing without the need for double-stranded DNA breaks (DSBs) or donor DNA templates. PEmax requires a unique long gRNA known as "pegRNA" shown schematically in Figure 1.

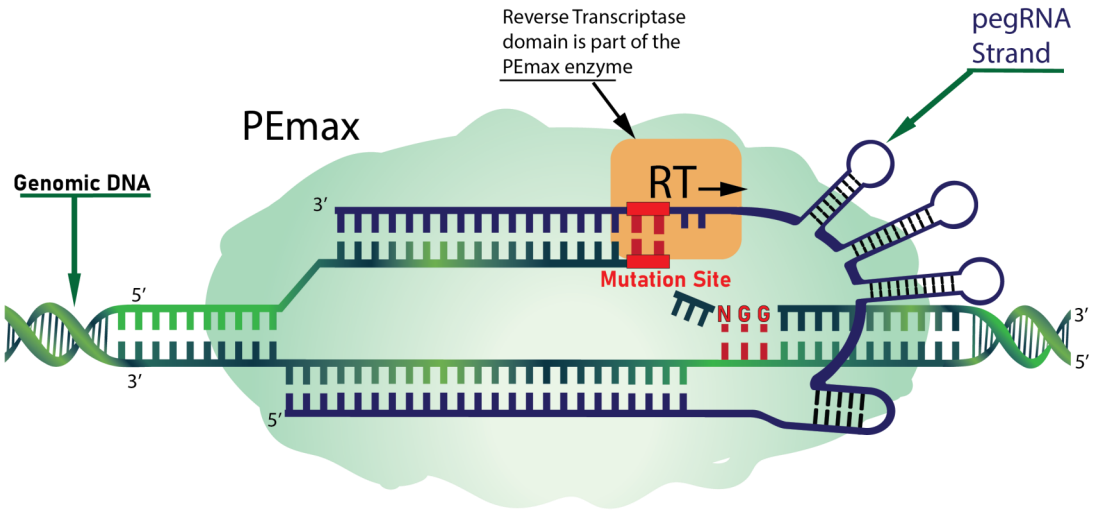

Fig. 1: Overview of the CRISPR-associated (Cas) system showing a representative prime-editing enzyme oriented with target genomic DNA and pegRNA.

Intact Genomics (IG®) PEmax enzyme is the purified recombinant Streptococcus pyogenes Cas9 nickase mutant (H840A)-Murine Moloney Leukemia Virus-Reverse Transcriptase (MMLV-RT) fusion protein[3-4] containing a nuclear localization signal (NLS) at the C-terminal for targeting to the nucleus. This enzyme is designed to perform CRISPR prime-editing[3-4]. The physical purity of this enzyme is ≥85% as assessed by SDSPAGE with Coomassie® blue staining.

Product Source:

E. coli BL21 (DE3) strain expressing a recombinant PEmax gene from *Streptococcus pyogenes* with custom protein purification tags.

Components and Storage:

PEmax Enzyme Kit contains the items below. Store all components at -20°C.

- PEmax enzyme
- 10x PEmax Reaction Buffer

Storage Buffer:

- 50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.5 @ 25 ºC

1x Cas9 Reaction Buffer:

- 20 mM HEPES, 100 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA, pH 6.5 @ 25 °C

Quality Control:

PEmax enzyme is free from detectable RNase, Endonuclease (nicking) and non-specific DNase activities.

Functional Testing:

We have demonstrated that PEmax has specific nicking activity in the presence of an *in vitro* transcription-synthesized pegRNA guide molecule (Fig.2) and *in vitro* RT activity (not shown).

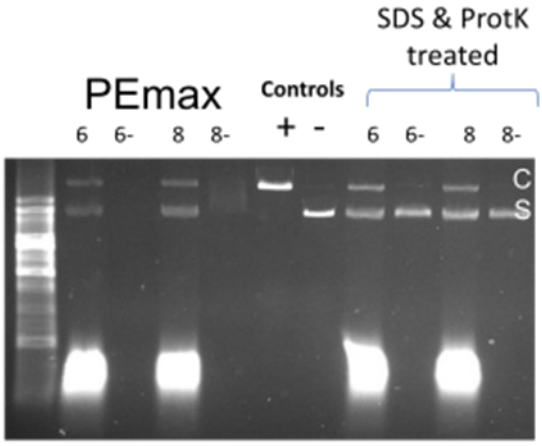

Figure 2. PEmax successfully nicks DNA, and it requires pegRNA for function. The PEmax nicking assay shows circular (C) and supercoiled (S) dsDNA. The positive (+) control uses Nb.BssSI, a nicking restriction enzyme from NEB, which nicks DNA so that it runs mostly as circular DNA (lane +). The negative (–) control is dsDNA with NO enzyme added. The DNA is mostly supercoiled; the circular DNA is too faint to see (lane –). PEmax is unable to nick without pegRNA (lanes 6- and 8-). However, in the presence of pegRNA, PEmax nicks dsDNA generating more circular dsDNA (lanes 6 and 8). For better visibility of resulting nicked DNA, SDS and Proteinase K were added to the reaction sample to remove proteins so that more DNA could enter the gel.

Related Products:

- IG® PEmax pegRNA synthesis kit (Cat.# 3403, 3406)
- IG® RNA Cleanup Kit (Cat.# 4003, 4005)
- IG® sgRNA Synthesis Kit for Cas9 Nuclease (Cat.# 3203, 3206)
- IG® sgRNA Synthesis Kit for Cas12a Nuclease (Cat.# 3303, 3306)
- Cas9 Nuclease (Cat.#3273, 3276)
- Cas12a Nuclease (Cat.# 3373, 3376)
- ig® 10B Chemically Competent Cells (Cat.# 1011-12)

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- Order online within the USA. Place orders on **www.intactgenomics.com** using our secure Shopping Cart.
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  Email: **sales@intactgenomics.com**
  Phone: (314) 942-3655 **|** Toll-free : 855-835-7172 **|** Fax: (314) 942-3656
- Order via our distributors.

References:

1. Jinek, M. et al. A programmable dual RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337, 816–821 (2012).

2. Mali, P. et al. RNA-Guided Human Genome Engineering via Cas9. Science 339, 823–826 (2013).

3. Chen, P. J. et al. Enhanced prime editing systems by manipulating cellular determinants of editing outcomes. Cell 184, 5635-5652.e29 (2021).

4. Anzalone, A. V. et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 576, 149–157 (2019).

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