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Assay method of PfAgo endonuclease activity

PfAgo核酸内切酶活性检测方法

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Foreword

This document is in accordance with the provisions of GB/T 1.1-2020 "Guidelines for standardization work Part 1: Structure and drafting rules for standardization documents" Drafting.

Please note that some of the contents of this document may involve patents. The issuing organization of this document does not assume the responsibility for identifying patents.

This document was proposed and coordinated by the National Tool Enzyme Standardization Working Group (SAC/SWG11).

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Introduction

PfAgo endonuclease can cleave the DNA guide strand (greater than 15 nt) under the guidance of the 5' phosphorylated DNA guide strand.

The target DNA to be complemented, the cleavage site is generally at the 10th and 11th bases corresponding to the target DNA starting from the 5' end of the DNA guide strand The cleavage function of PfAgo endonuclease is not limited by the target DNA and the adjacent base sequence of the cleavage site.

The activity of PfAgo endonuclease is relatively high and it also has some activity against target RNA substrates.

The establishment of national standards to promote their industrialization is of great significance for the production and use of this type of tool enzymes.

PfAgo endonuclease activity detection method

1 Scope

This document describes a method for detecting PfAgo endonuclease activity.

This document is applicable to the detection of PfAgo endonuclease activity.

2 Normative references

GB/T 191-2008

GB/T 6682

3 Terms and Definitions

The following terms and definitions apply to this document.

3.1 PfAgoendonuclease

Based on the complementary base pairing between the DNA guide strand (gDNA) sequence and the target DNA (tDNA) sequence Yes, then the endonuclease that specifically recognizes and cuts the target DNA.

3.2

In a 25 μL reaction volume, at 95°C, the amount of enzyme required to cleave 0.1 nmol of target DNA per minute is one activity unit (U).

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4 Principles

Based on the complementary base pairing between the DNA guide strand sequence and the target DNA sequence, PfAgo endonuclease can phosphorylate the The target DNA complementary to the DNA guide strand is cut under the guidance of the DNA guide strand (greater than 15 nt). The cutting site often occurs at the end of the DNA guide strand.

The guide strand is complementary to the target DNA at the phosphodiester bond between bases 10 and 11 of the target DNA.

5.1 Water

Grade II water in accordance with GB/T 6682. 5.2 1mol/LTris-HCl (pH 8.0) Weigh 12.114 g of Tris, add it to 80 mL of ultrapure water, mix thoroughly, adjust the pH to 8.0 ± 0.05 with 6 mol/L hydrochloric acid, and add Add ultrapure water to a volume of 100.0 mL and filter with a 0.22 μm microporous filter membrane. Store at 2°C to 8°C. The shelf life is 24 months.

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