Tiosbio® CRISPR Double Enzyme Cutting Nucleic Acid Detection Strip (Chameleon)

Cat. No. JY0308

Working Mechanism:

This product uses a chromatographic double-antibody sandwich method to detect the enzyme cleavage products of Cas12 and Cas13. After the target gene is amplified by LAMP, RAA/RPA, and PCR, use Cas12 and Cas13 enzymes to cut the amplified product at the same time, and the DNA signal probe A corresponding to the Cas12 enzyme (Cas12 cleavage), or the RNA signal probe corresponding to the Cas13 enzyme Needle B (Cas13 cleavage) is modified as follows, then this product can be used for detection of cleavage products:

1) One end of probe A is modified with biotin (Biotin), the other end is modified with fluorescein isothiocyanate (FITC) or 6-carboxyfluorescein (6-FAM), and one end of probe B is modified with biotin (Biotin) , the other end is modified with digoxin (Dig);

2) Or, one end of probe A is modified with biotin (Biotin), the other end is modified with digoxin (Dig), and one end of probe B is modified with biotin (Biotin), and the other end is modified with fluorescein isothiocyanate (FITC) or 6-carboxyfluorescein (6-FAM).

Intended Use:

Detection of products after Cas12 and Cas13 double digestion.

Packing specification/Article number:

Packing specification：50T/pack, packed in moisture-proof aluminum foil bags.

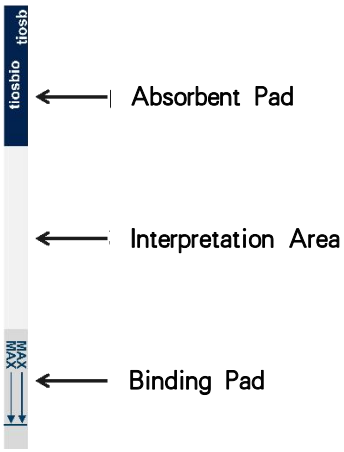

Figure 1 Schematic diagram of the structure of a disposable nucleic acid detection test strip

Storage conditions and expiration date:

Storage conditions: avoid light and moisture, at a temperature of 4~30°C.

Expiry date: 12 months from production.

Instruction:

1. **Take out the corresponding number of test strips according to the number of test samples, and mark them on the absorbent pad (Figure 1). Each test strip can only be used for an one-time single sample test. When the volume of the amplification product is between 50-100μL, the nucleic acid product can be detected directly in the 200μL PCR reaction tube. When the amplification product is less than 50μL, it is necessary to add ultrapure water to the**

PCR tube to make up the volume to 50μL, and the detection can only be performed after mixing by pipetting.

2. After PCR, RPA or RAA products are added to the CRISPR system, open the PCR reaction tube and insert the binding pad end (arrow end) of the test strip into the PCR reaction tube (Figure 1). The liquid level must not exceed the top of the binding pad. After the reading area is fully saturated (about 1-2 minutes, when the external environment temperature is low, such as winter, the water absorption speed will be reduced, and the saturation time of the reading area will be prolonged), and after the color of the quality control line (C line) emerges, take the test strip out. Read the test result directly according to the color development of the test strip.
3. Read the results within 10 minutes after the color of the quality control line (C line) emerges. Interpretation after 10 minutes is invalid.
4. Record the test results, seal and dispose of the test strips in a safe place.

Interpretation of results:

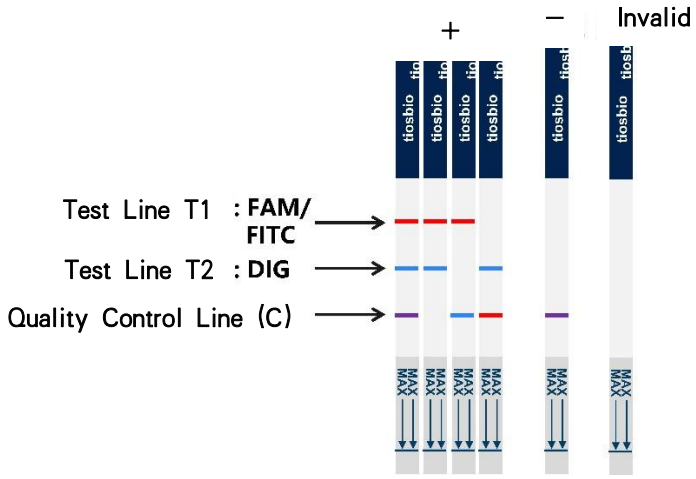

Figure 2 Interpretation of CRISPR double-enzyme nucleic acid detection test strip results

Color explanation: This product is a color-changing double-correction color test strip, in which the C line is purple, T1 is red, and T2 is blue. When probe A and probe B are mixed at equimolar concentrations, and the final concentration is ≤500nM, after digestion of the FAM-labeled probe, the color of T1 will be red, and the C line will gradually turn blue; After digestion of the probe, the color of T2 is blue, and the C line gradually turns red, so as to achieve the purpose of mutual correction.

1. **Positive（+）:**

Bands appear on both the test strip quality control line (C line) and the two test lines (T line), among which T1 is red, T2 is blue, and C line is purple; the test strip quality control line (C line) does not show color, when T1 is red and T2 is blue. Both indicate that Cas12 and Cas13 can carry out effective enzyme digestion, and activate the reporter group to develop color, and the corresponding target genes can be regarded as positive.

Bands appear on the test strip quality control line (C line) and one of the detection lines (T line), among which: T1 is red and C line is blue, indicating that the corresponding target gene of the FAM-labeled signal molecule can be judged as positive; T2 is blue, and C line is red, indicating that the corresponding target gene of the Dig marker molecule can be judged as positive.

试纸条质控线（C 线）和其中 1 条检测线（T 线）出现条带，其中：T1 为红色，C 线为蓝色时，说明 FAM 标记信号分子的相应靶基因可判定为阳性；T2 为蓝色，C 线为红色时，说明 Dig 标记分子的相应的靶基因可判定为阳性。

2. **Negative（-）:**

A purple line emerges on the test strip quality control line (C line), and the test line (T line) does not develop color,

which is judged as a negative result. This result indicated that neither Cas12 nor Cas13 could cut the reporter molecule, and failed to activate the reporter group for color development.

3. **Invalid：**

   There are no bands on the quality control line (C line) or test line (T line) of the test strip, indicating that the test strip or amplification reagent used may be damaged, invalid or the user has not followed the instruction. In these cases, read the instructions carefully, and amplification and detection should be reconducted. If the problem persists, stop using the product with the same batch number immediately and contact the local supplier.

Precautions and Safety Tips:

1. **This product should be used in tandem with probes. If the purity of the probe synthesis is insufficient, that is, when the probe contains free Biotin or free FITC, the T line of the cleaved product that uses ultrapure water as a negative control will appear red, which produces a false positive result.**
2. **This product can be used to test the quality of probe synthesis. Adjust the concentration of the probe used in the blank negative control to 400 nM, and perform a cleavage reaction. If the T line of the test strip appears red within 5-7 minutes after immersing the end of the test strip binding pad in the Cas12/Cas13 cleavage product, then the purity of the probe cannot meet the experimental requirements, and false positive results may be caused by insufficient probe purity. It is recommended to change the probe synthesis supplier and re-synthesize the probe.**
3. **This product is for scientific research use only. Please read the instructions carefully before use and operate in strict accordance with the instructions. Violation or failure to follow instructions may result in erroneous results.**
4. **The product should be stored in a suitable environment and temperature according to the instructions, and used before the expiry date. Improper storage or expired product may lead to erroneous results. Please use the test strips as soon as possible after opening the package, so as not to affect the test results due to the dampness of the test strips. Insufficient light in the detection environment, an operator who suffers from color weakness and other factors may also lead to wrong results.**
5. **After use, put the test strip into a sealed bag as soon as possible and dispose of it properly. This product is for one-time use only, please do not reuse it.**