Working Principle:

This product uses lateral flow dual-antibody sandwich assay to simultaneously detect target gene CRISPR enzyme cleavage products and LAMP, RAA, RPA, or PCR amplification products. When CRISPR signal probe (A) and amplification product (B) are modified as follows, this product can be used for detection—CRISPR signal probe (A) is modified with Biotin at one end and FITC or 6-FAM at the other end; amplification product (B) is modified with Digoxin (Dig) at one end and Rhodamine (TAMRA) at the other end.

Intended Use:

Simultaneous detection of CRISPR single-cleavage products and amplification products.

Package Specification/Cat. No.:

Package Specification: 50 strips/tube, aluminum foil bag moisture-proof packaging.

Figure 1. Schematic Diagram of Disposable Nucleic Acid Detection Test Strip Structure

Storage Conditions and Shelf Life:

Storage Conditions: Store in a dark and dry place at 4-30° C.
Shelf Life: 12 months.

Procedure:

1. Take out the corresponding number of test strips according to the number of samples to be tested, and mark them on the absorbent pad (Figure 1). Each test strip can only be used for single detection of 2 different products. When the CRISPR single-cleavage product volume is 50-100 μL, the nucleic acid product can be directly detected in a 200 μL PCR reaction tube. When the product volume is less than 50 μL, ultrapure water needs to be added to the PCR tube to make up the volume to 50 μL, mix thoroughly by pipetting, and then detection can be performed.
2. After the CRISPR enzyme cleavage reaction of LAMP, RAA/RPA, or PCR amplification products is completed, open the PCR reaction tube and insert the conjugate 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 conjugate pad. Wait for the reading zone to be fully wetted (approximately 1-2 minutes; when the ambient temperature is low, such as in winter, the wicking speed will be reduced and the wetting time of the reading zone will be extended). After the test line (T line) develops color, the test strip can be removed. Read the detection result directly according to the color development of the test strip.

3. Observe the results within 10 minutes after the control line (C line) develops color. Reading after 10 minutes is invalid.
4. Record the detection results and seal and discard the test strips in a safe place.

Interpretation of Results:

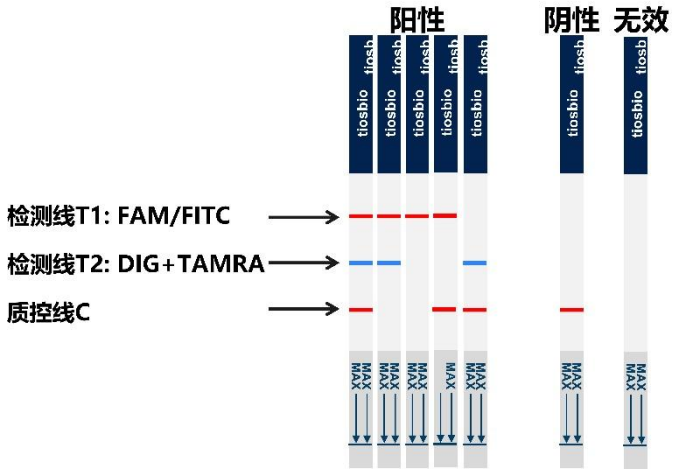

Figure 2. Schematic Diagram of CRISPR Single-Cleavage and Amplification Product Detection Test Strip Result Interpretation

1. Positive (+):

Bands appear at both the control line (C line) and the 2 test lines (T line) of the test strip, where T1 is red, T2 is blue, and C line is red; when the test strip control line (C line) does not develop color, T1 is red, and T2 is blue, it indicates that the CRISPR system can perform effective cleavage and activate the reporter group to develop color, and the corresponding target genes can all be judged as positive.

A band appears at the control line (C line) and one of the test lines (T line) of the test strip, where T1 is red and C line is red, or only T1 is red, it indicates that the CRISPR system can perform effective cleavage and activate the reporter group to develop color, and the corresponding target gene can be judged as positive; when T2 is blue and C line is red, it indicates that the target gene amplification product labeled with Dig and Rhodamine can be judged as positive.

2. Negative (-):

The control line (C line) of the test strip shows a red band, and the test line (T line) does not develop color, which is judged as a negative result.

3. Invalid:

No bands appear at both the control line (C line) and the test line (T line) of the test strip, indicating that the test strip or amplification reagent used may be damaged, invalid, or there was an operational error. In this case, read the instructions carefully, re-amplify and re-test. If the problem persists, stop using the product from the same batch immediately and contact the local supplier.

Warnings and Precautions:

1. This product should be used in combination with probes. If the probe synthesis purity is insufficient and the probe contains free Biotin or free FITC, it will cause the T line of the cleavage product with ultrapure water as negative control to appear red, that is, the negative control shows a false positive result.
2. This product can be used for probe synthesis quality testing. Adjust the probe concentration in the blank negative control to 400 nM and perform the cleavage reaction. When the test strip conjugate pad end is immersed in the Cas12/Cas13 cleavage product for 5-7 minutes, if the test strip T line appears red, it indicates that the probe purity is difficult to meet the experimental requirements, and false positive results

will occur due to insufficient probe purity. It is recommended to change the probe synthesis supplier and resynthesize the probe. When the probe concentration of this product is 20-50 nM, the T line will not develop color within 30 minutes after the test strip conjugate pad end is immersed in the Cas12/Cas13 cleavage product.

3. **This product is for research use only. Please read the instructions carefully before use and operate strictly according to the instructions. Violation or failure to operate according to the instructions may lead to erroneous results.**
4. **The product should be stored under appropriate environmental conditions and temperature according to the instructions and used within the validity period. Improper storage or expired product may lead to erroneous results. Use the test strips as soon as possible after opening the package to avoid affecting the test results due to moisture. Insufficient lighting in the detection environment, operator color weakness, and other factors may lead to erroneous results.**
5. **After use, put the test strips into a sealed bag as soon as possible and dispose of them properly. This product is for single use only. Do not reuse.**