Working Principle:

This product uses lateral flow dual-antibody sandwich assay to detect Cas12 and Cas13 enzyme cleavage products. After the target gene is amplified by LAMP, RAA/RPA, or PCR, Cas12 and Cas13 enzymes are used to simultaneously cleave the amplification products. The DNA signal probe A corresponding to Cas12 enzyme (Cas12 cleavage) and the RNA signal probe B corresponding to Cas13 enzyme (Cas13 cleavage) are modified as follows to use this product for cleavage product detection:

1） Probe A is modified with Biotin at one end and FITC or 6-FAM at the other end, Probe B is modified with Biotin at one end and Digoxin (Dig) at the other end;

2） Or, Probe A is modified with Biotin at one end and Digoxin (Dig) at the other end, Probe B is modified with Biotin at one end and FITC or 6-FAM at the other end.

Intended Use:

Detection of products after Cas12 and Cas13 dual-enzyme cleavage.

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 products from simultaneous cleavage of DNA probe and RNA probe. When the enzyme 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 system enzyme cleavage reaction is completed for LAMP, RAA/RPA, or PCR amplification products, 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:

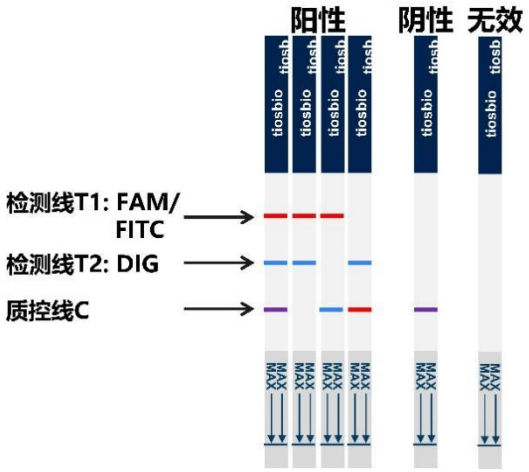

Figure 2. Schematic Diagram of CRISPR Dual-Enzyme Nucleic Acid Detection Test Strip Result Interpretation

Color Explanation: This product is a color-changing dual-correction colorimetric test strip, where C line is purple, T1 is red, and T2 is blue. When probe A and probe B are mixed at equimolar concentration with a final concentration ≤500 nM, after the probe labeled with FAM is enzymatically cleaved, T1 appears red and C line gradually turns blue; after the probe labeled with Dig is enzymatically cleaved, T2 appears blue and C line gradually turns red, thereby achieving mutual correction.

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 purple; when the test strip control line (C line) does not develop color, T1 is red, and T2 is blue, it indicates that Cas12 and Cas13 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: when T1 is red and C line is blue, it indicates that the corresponding target gene of the FAM-labeled signal molecule can be judged as positive; when T2 is blue and C line is red, it indicates that the corresponding target gene of the Dig-labeled molecule can be judged as positive.
2. **Negative (-):**

   The control line (C line) of the test strip shows a purple band, and the test line (T line) does not develop color, which is judged as a negative result. This result indicates that neither Cas12 nor Cas13 can cleave the reporter molecule and fails to activate the reporter group to develop color.
3. **Invalid:**

   No bands appear at both the control line (C line) and the 2 test lines (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 will cause false positive results due to insufficient probe purity. It is recommended to change the probe synthesis supplier and resynthesize the probe.**
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.**