CRISPR-Cas12a DNA detection kit (2-step)(lyophilized)(paper strip)

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Catalog Code:D-L-CAS12-LYO-2S

CONTENTS

Contents	Page

Brief introduction ... 1

Materials supplied ... 1

Other materials required ... 1

Storage ... 2

Sample for detection ... 2

Assay procedure ... 2

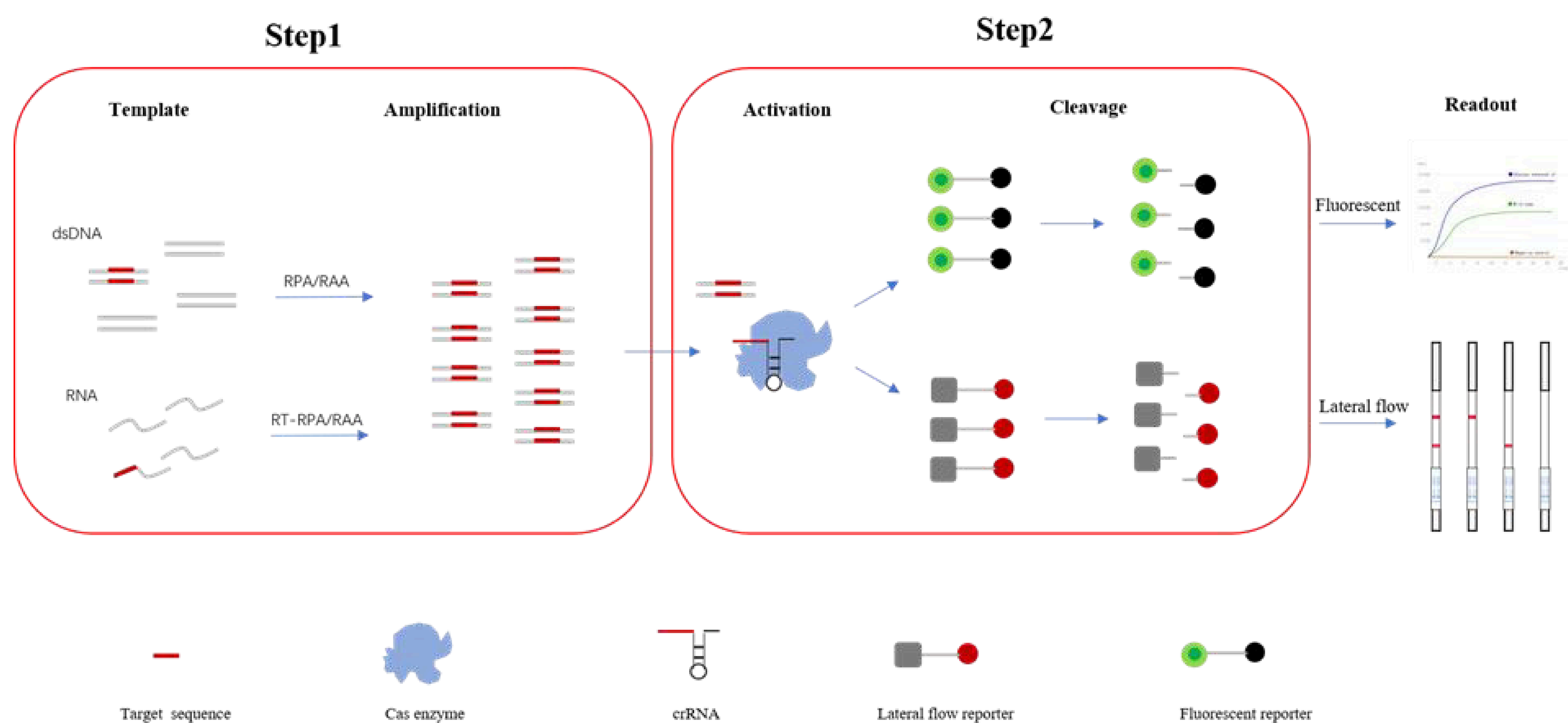
Interpretation of results ... 4

Schematic Diagram of Principle ... 5

Notes ... 6

Brief introduction

This kit combines isothermal amplification and CRISPR/Cas12a to achieve sensitive and specific DNA detection. This kit contains the universal reagents for both the isothermal amplification and the CRISPR/Cas12a detection. It makes R&D of point-of-care products easily.



Materials supplied

#	Component	Volume
1	Reaction Tube	96T
2	Reaction Buffer (2X)	1000 μl
3	Starter (10X)	200 μl
4	Cleavage Buffer （10X)	240 μl
5	Diluent	8000 μl
6	Cas12a Protein_ lyophilized	Add PCR water according to the label on the tube before use.
7	Cas12a Reporter_ lyophilized	
8	Positive Control_ lyophilized (primer and DNA template included)	
9	crRNA for Positive Control_ lyophilized	

Other materials required

i. Lateral flow paper strip that detects amplicon with dual labels of FAM and biotin. Recommend EZassay lateral flow paper strip (indirect method for CRISPR SHERLOCK). Catalog number: CS-FMBO-96.
ii. Template DNA
iii. RPA/RAA primers (online software: https://www.ezassay.com/primer)
iv. Q-PCR thermal cycler or heat block or water bath
v. Nuclease-free water
vi. crRNA/gRNA for LbaCas12a.
LbaCas12a crRNA scaffold sequence: 5’ - UAAUUUCUACUAAGUGUAGAU-3’

LbaCas12a crRNA

A
A G
U U
C-G
A-U
U-A
C-G

Scaffold sequence (conserved)

Guide sequence (20~23nt)

5'-UAAUUU-AU AAAUAUGACAUCAUGGUAACAAA

3'-NNNNNNNNNNNNNNNNNAAABTTTATACTGTAGTACCATTGTTTNNNNNNNNNNNNNNNN-5'
5'-NNNNNNNNNNNNNNNNNTTTV AAATATG ACAT CAT GGTAACAAANNNNNNNNNNNNNNNN-3'

B for G/T/C
V for G/A/C

PAM

Target sequence

Storage

-20°C. Suggest aliquot. Avoid repeated freeze-thaw.

Assay procedure

1. The first part is amplification. Set the working temperature at 39 °C. Turn off the lid heating function or set to 42 °C if PCR thermal cycler is used.

● Add PCR water according to the label on the tubes and mix well.

Note. Before opening the lid, it is suggested to centrifuge the reaction tube at 3000-4000 rpm for 1 minute to collect reagents.

● For each reaction tube, add reagents as described in the table below. Suggest to set up on ice.

#	Component	Volume
01	Reaction Buffer (2X)	10 μl
02	Forward Primer (20μM) Reverse Primer (20μM)	0.5μl 0.5μl
03	DNA template*	x μl
04	Starter (10X) **	2 μl
05	Nuclease-free H2O	To a total volume of 20 μl

*For no template control group, use Nuclease-free water. For positive control group, add 2 μL Positive Control as provided in the kit.
** Add starter at the final step and mix thoroughly.

- Flick to mix, quick spin down. Repeat 3 times.
- Incubate at 39°C for 20~40 minutes.
- Run agarose gel to make sure the amplification works.

2. The second part is CRISPR/Cas12a detection. Set the working temperature at 37 °C.

- Take 20μl reaction volume as an example. Prepare reactions as described in the table below. Suggest set up the reaction on ice.

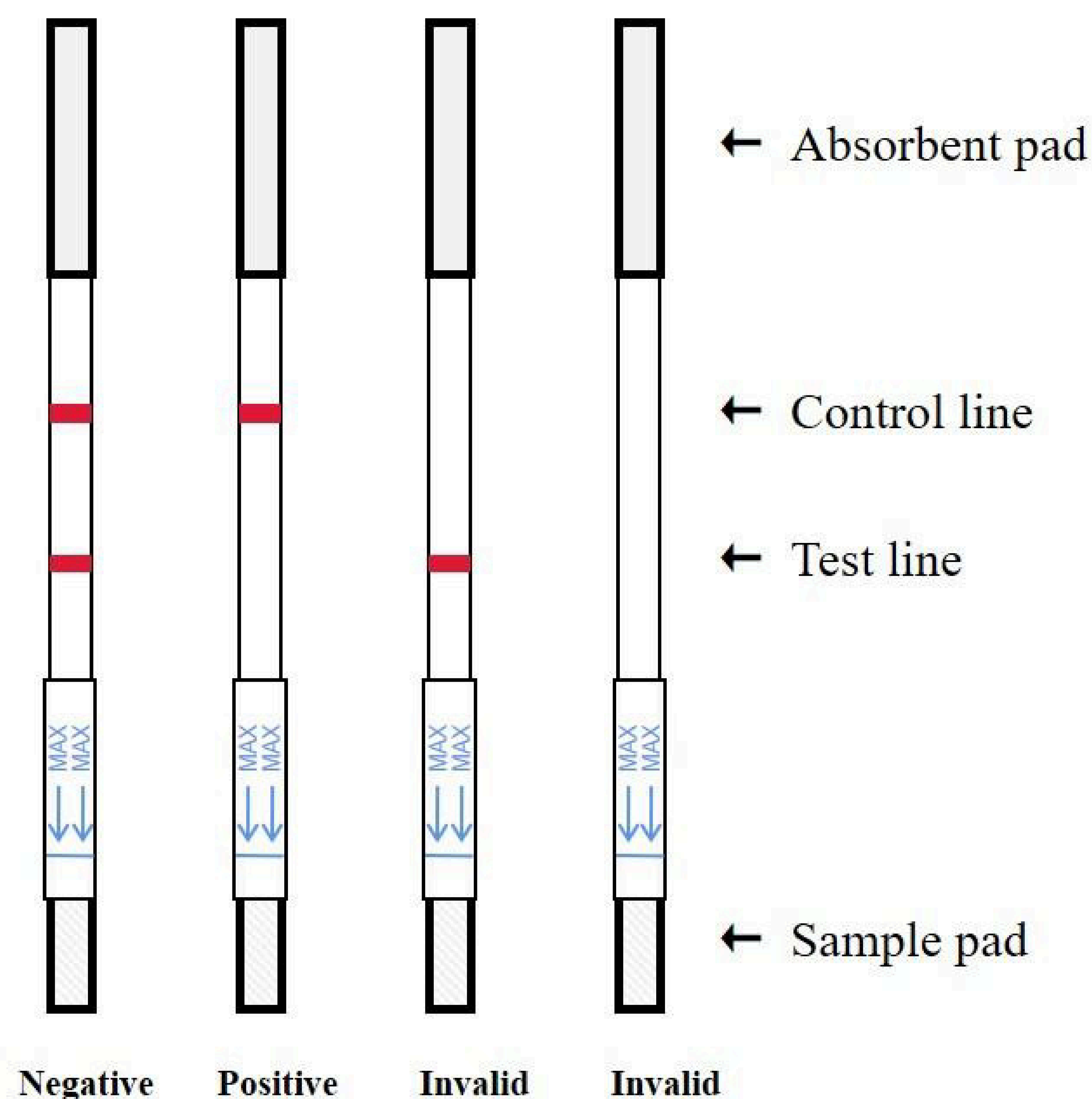
#	Component	Volume
01	Cleavage Buffer （10X）	2 μl
02	Cas12a Reporter (4μM)	0.25 μl
03	Cas12a Protein （1μM） *	1 μl
04	crRNA (Cas12a) (1μM) **	1 μl
05	Amplicons from last step ***	x μl
06	Nuclease-free H2O	To a total volume of 20 μl

* Diluent Cas12a Protein properly with ddH2O before use.
**The positive control group adds 1μl of crRNA for Positive Control (20X). For the other group, add the specific crRNA for sequence of interest.
*** x ⩽ 5μL.

- Flick to mix, quick spin down. Repeat 3 times. Incubate tube at 37 °C for 30 ~ 60 minutes.

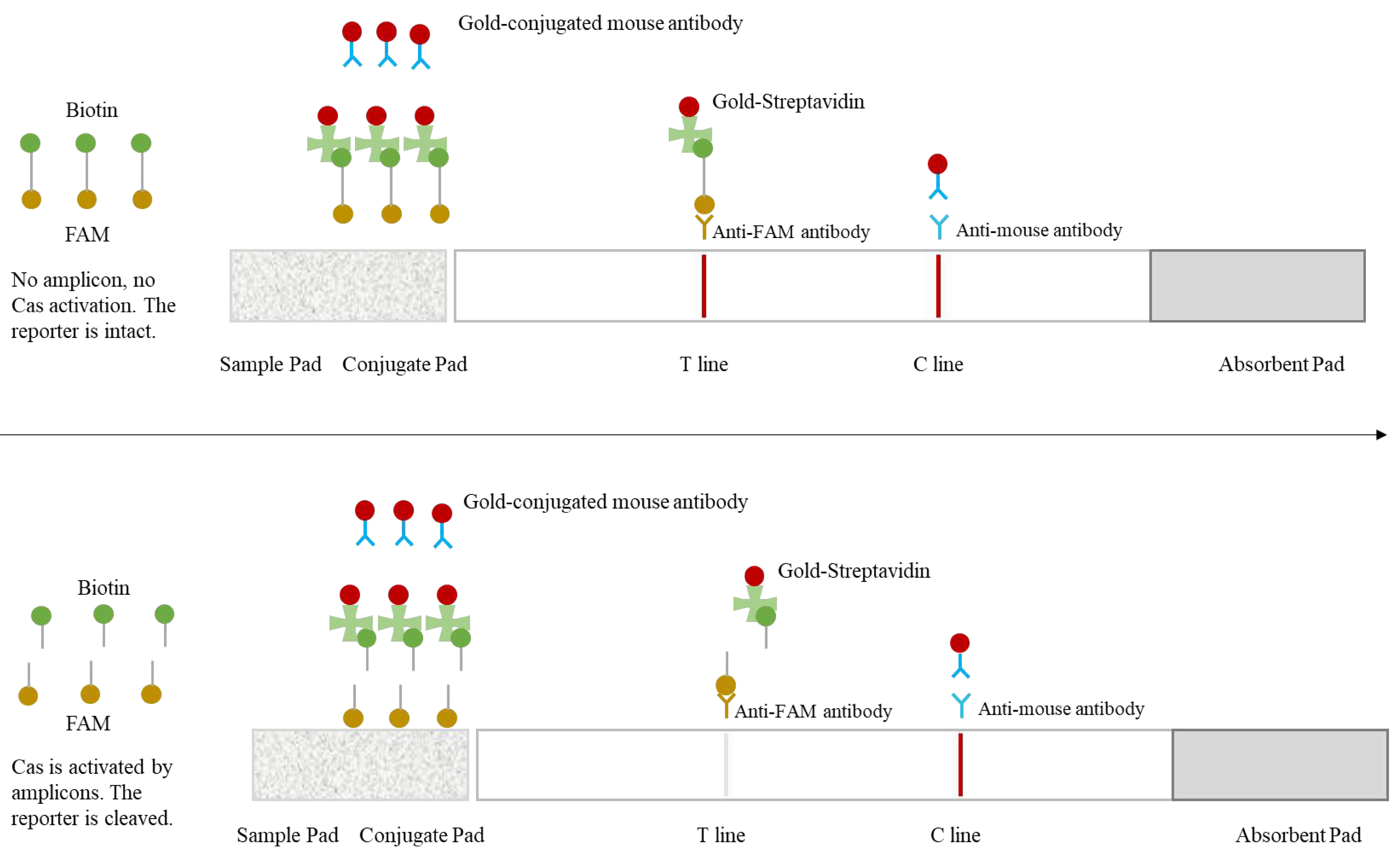
3. Observe the result.
- Take 1~10μL of the product and dilute it 9~200 folds with Diluent. (e.g. Add 2uL product into 78uL Diluent.)
- Take 70μL of the dilution and add it to paper strip, and observe the result within 5 minutes.

Interpretation of results



Lateral flow paper strip (indirect method for CRISPR SHERLOCK)
Catalog number: CS-FMBO-96.

Schematic Diagram of Principle



Attention

l Designate and use distinct areas for sample preparation, reaction setup, and analysis to avoid carry-over contamination.

l Do not open the tube after amplification if possible. Please enquiry EZassay team for technical support.

l If PCR thermal cycler is used, please make sure to turn off the heat lid function or set to 40 °C.

l The optimal concentration of primer, Cas protein, and crRNA varies depending on the projects. Optimized range of working concentration: primers: from 300nM~800nM for each primer; crRNA: 20nM~1000nM; reporter: 20nM~1000nM; Cas12a: 20nM~200nM.

Professional supplier of point-of-care test products

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