Reagent

apt-1 (100μM)

apt-2 (100μM)

activator (100μM)

Cas12a (6nM in buffer A)

crRNA (3nM in RNase free buffer)

ssDNA-FQ (50nM, in RNase free buffer)

Kanamycin

Buffer A pH7.4 40 mM HEPES, 100 mM NaCl, 20 mM MgCl2

Preparation of solutions

Solution 1-probe solution 80μL

Reagent	Volume/μL
apt-1 4×	20
apt-2 4×	20
Activator 8×	10
Buffer A	30

The concentrations of the three oligonucleotides in the solution are 25nM,25nM and 12.5nM, respectively.

1.Incubate at 90℃ for 10min

2.slow cooling on the benchtop

Solution 2-reporter solution 120μL

Reagent	Volume/μL
Cas12a (6nM in buffer A)	40
crRNA (3nM in RNase free buffer)	40
ssDNA-FQ	40

1.Mix 40μL Cas12a (6nM in buffer A) and 40μL crRNA (3nM in RNase free buffer) and wait for 30 minutes at 25℃

2.Add 40μL ssDNA-FQ (50nM) to the mixture

Solution 3-kanamycin

120μM kanamycin solution

Detection

Solution/μL Group	1 Control	2 (60μM kana)	3 (120μM kana)
S1	20		
S2	20		
S3	0	20	40
Buffer	40	20	0
Total volume	100	100	100

Each tube:

1.Mix Buffer with Solution 3

2.Mix 20μL solution 1 (probe) and the mixture got from step 1. Incubate for 5 min.

3.Add 60μL solution 2 (reporter) and incubate at 25℃ for 35 min.

4.Incubate at 60℃ for 5 min (stop enzymatic reaction)

5.Detect the fluorescence intensity (the excitation wavelength is 485nm and the emission was monitored at 520nm)