Fluorescence Based Nucleic Detecting Assay

Aim of the experiment

1. Characterize the activity of the purified protein, and measure the fluorescence intensity time curve to provide experimental data for quantitative modeling;

2. Set different virus concentration experiments to get the lowest limit of virus detection concentration;

3. Verify the cutting preference of cas13 proteins to probe

Materials

• Reagents:

nuclease-free H2O (nf H2O）

RNase inhibitor Murine （40U/uL）

Fluorescence reporter（ortholog's sequence-specific reporter)

1M MgCl2(RNase free)

Cas13 protein orthologs(LwaCas13a protein,LbaCas13a protein,PsmCas13b protein,CcaCas13b protein)

crRNAs(designed based on different Cas13 proteins)

Viral target(55nt of SARS,SARS-COV-2,MERS,H1N1)

RNase Away (Thermo Fisher Scientific, cat. no. 10328011)

2.5 mL of Tris-HCl (pH 7.5, 1 M)

6 mL of NaCl (5 M)

Protein storage buffer[2.5 mL of glycerol,100uL DTT(1M),Ultrapure water up to 50mL,Filter through a 0.22-μm,50-mL vacuum filter,Stored at 4℃ at 48h,-20℃ for up to a year storage.]

• Equipment:

384-well, round black-well plate

fluorescence plate reader(TECAN Spark)

Procedure

Table 1: the components and the groups of the detecting

components/groups	blank(uL)	Negative 1(uL)	Negative 2(uL)	Experimental group (uL)
RNase free H2O	Up to 80	Up to 80	Up to 80	Up to 80
HEPES(1M)	1.6	1.6	1.6	1.6
MgCl2(1M)	0.8	0.8	0.8	0.8
Cas13a protein	1.44	1.44		1.44
crRNA	9	9	9	9
RNase inhibitor	10	10	10	10
Fluorescence reporter		5	5	5
Target				

Tips: The volume of the mix is for 4 replicates; for multiplexing applications,add 5 uL to each Fluorescence reporter; the amount of target is based on specific experiments.

Table 2: The excitation,emission wavelengths, fluorophores and quenchers of different fluorescent reporters

Cas13 proteins	Fluorophore	sequence	Quencher	Absorbance (nm)	Emission (nm)
LwaCas13a	CY5	T*A*rUrCG*C*	BHQ-2	635	682
LbaCas13a	FAM	T*A*rArCG*C*	BHQ-1	470	525
CcaCas13b	VIC	T*A*rArUG*C*	BHQ-1	523	564
PsmCas13b	Texas red-X	T*A*rGrAG*C*	BHQ-2	571	612

1. Use RNase away to wipe the clean bench and the consumables needed for the experiment;

2. Thaw sufficient amounts of crRNA,fluorescence reporters and Cas13 protein aliquot on ice, covered with aluminum foil to protect from light exposure. Dilute these materials to the corresponding concentration (Cas13 protein:.2.25uM,crRNA: 0.5uM,fluorescence

reporter:5uM)Sufficient amounts are calculated on the basis of the number of desired reactions, with a minimum 15% excess.

3. Preheat the fluorescence plate reader to 37 ℃, the oven to 65 ℃, and the incubator to 37 ℃

4. According to table 1,mix nfH2O, nuclear assay buffer (HEPES, MgCl2) and crRNA in Eppendorf tube and incubate it at 65 ℃ for 5 min (incubator) to remove the secondary structure(We recommend routinely performing four technical replicates per condition.)

5. Add Cas13 protein and incubate at 37 ℃ for 10 min to combine cas13 protein with crRNA, meanwhile incubate target at 65 ℃ for 5 min

6. Add reporter and RNase inhibitor, carefully open each Eppendorf tube and transfer 50 μL per technical replicate and condition to a 384-well,and add target of different concentrations before the measurement by the fluorescence plate reader (because the reaction starts with the addition of target).

7. Set up the procedure of fluorescence plate reader and read (The excitation and emission wavelengths of the corresponding fluorescent reporters are listed in Table 2)