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Characterization

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1 Works for me This protocol is published without a DOI.

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BEFORE STARTING

pH buffer preparing(in 15 ml centrifuge tubes):
pH2: 5 ml ddH2O, 8.8 ul 35% HCl, 37.5 mg KCl
pH5: 5 ml ddH2O, 20.8 ul 99.7% CH3COOH, 162 ul 3M CH3COOK, 31 ul 10N NaOH
pH7: 5 ml RIPA buffer, 1.5 ul 35%HCl
pH8: 5 ml RIPA buffer, 1 ul 10N NaOH
pH10: 5 ml ddH2O, 0.0147 g NaHCO3, 8.8 ul NaOH
pH12: 5 ml ddH2O, 32.4 ul 10N NaOH, 0.06 g KCl

In vitro protein synthesis

1 Add the reagents(on ice) for synthesis of mRFP into one well of the 384-well plate. Add the reagents from top to down.

Order	Location	Reagent	Amount
1	Upper-left	DNase/RNase free water	Till 30 µL
2	Upper-left	Solution A	12µL
3	Lower-right	Solution B	9µL
4	Lower-right	RNase inhibitor	1.2µL
5	Lower-left	RFP+terminator	300ng
		total	30µL

Do triple repeat

Pipetting Solution A, Solution B, RNase inhibitor before adding.
Pipetting the eppendorf contain RFP+terminator template to prevent precipitation.

2 Seal the plate with microseal (on ice).

3 Centrifuge **4000 rpm, 4°C, 00:01:00**

Setup plate reader 1

4 Plate type: 384 well plate
Select wells: At run time
Description: 1.Temperature **37 °C**

Incubation

5 Put the plate into plate reader.
Incubate the mixture and measure the fluorescence in plate reader follow Setup 1 for **02:00:00**

Addition of Buffer

6 Take the 384 well plate out of the plate reader.

7 Centrifuge **4000 rpm, 4°C, 00:01:00**

8 Remove its seal.

9 Apportion 5 µL of mRFP reagent into 18 well.

Pipetting before apportion.
We actually add 4 µL into every well since we think it may evaporate.

10 Add pH2,5,7,8,10,12 Buffer solution respectively into different wells of mRFP till total volume of 100 µL.
Do triple repeat.

Pipetting after adding buffer solution

11 Seal the well.

12 Centrifuge **4000 rpm, 4°C, 00:01:00**

Setup Plate Reader 2

13 Plate type: 384 well plate

14 Temperature: 37 °C

15 Excitation start: 550nm, Stop: 620 nm
Fixed emission: 650 nm
Step: by 1 nm
Gain: Auto Gain (measured with selected wells)

16 Emission start: 550nm, Stop: 700 nm
Fixed excitation: 540nm
Step: by 1 nm
Gain: Auto Gain (measured with selected wells)

Measuring

17 Measure the fluorescence excitation and emission intensity of mRFP