

S3.1 Layout of key components and basic properties of the Portable Fluorometer



① Micro-USB B cable port. ② Sample chamber. ③ Power indicator.

Fig. S1 Layout of key components of the Portable Fluorometer.

Table S1 The following table lists the physical, electrical and optical properties of the Portable Fluorometer:

Instrument	Portable Fluorometer
Dimensions	L x W x H (9.0 cm x 7.2 cm x 3.9 cm)
Weight	135 g
Dynamic Range	5 orders of magnitude
Light source	Blue LED (max ~470 nm)
Excitation Filter	Blue 457 - 487 nm
Emission Filter	Green 515 - 565 nm
Detector	Photodiode
Tube Type	0.2 mL clear, thin-wall PCR tubes
Minimum Assay Volume	150 μ L

It can be seen from **Table S1** that the fluorimeter (**Fig. S1**) has the palm size and portable. It is independently developed in the laboratory of Xi'an Runying Biotechnology Ltd. The device is simple, fast, accurate, and most importantly, it can be operated on site.

The following section details the steps required to run an assay using the Portable Fluorometer. 1. Follow the instructions to install the AAT Bioquest Device Cloud Software on your computer. 2. Once the Device Cloud software is installed and running on the local computer, use the Micro-USB B cable (**Fig. S1** ①) to connect the instrument to the computer. 3. Shortly thereafter, the power indicator (**Fig. S1** ③) light on the device lights up, indicating successful pairing with the Device Cloud software. 4. Once the device is successfully paired with the Device Cloud software, the prepared PCR tube containing the test sample can be placed into the sample chamber (**Fig. S1** ②). 5. Once all samples have been read, the data can be exported into Microsoft Excel for further analysis.

S3.2 Fluorescence intensity stability and dynamic range of the fluorometer

The excitation and emission detection wavelengths of the independently developed hand-held fluorometer are in the range of 457-487 nm and 515-565 nm respectively. The excitation and emission detection wavelengths were designed to match the spectra of fluorescein. The testing results are shown in **Fig. S2**.

It can be seen from **Fig. S2A** that there was an excellent linear relationship between the values of relative fluorescence intensity (VRFIs) and the concentrations of fluorescein ($R^2=0.9992$). The results revealed that the fluorometer can accurately respond to the VRFIs of fluorescein within 1-2500 nmol/L. When the concentration of fluorescein was below 1 nmol/L, the VRFIs showed irregular fluctuations with the decrease of the luciferin concentration, and the relationship between VRFIs and concentrations was not linear (**Fig. S2B**). While the fluorescein concentration was above 2500 nmol/L, the VRFIs reached to the maximum respond value of the fluorometer (**Fig. S2A**). Therefore, the concentrations of fluorescein were below 1 nmol/L or above 2500 nmol/L, the VRFIs are not accurate. The results showed that the dynamic range of VRFIs of the fluorometer are within 45 to 30133 nmol/L.

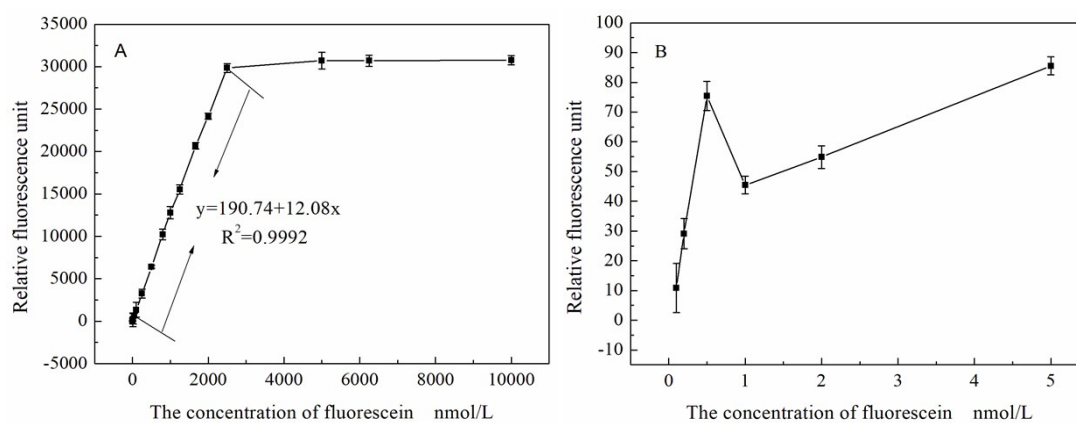


Fig. S2 Relationship between the VRFIs and the concentrations of fluorescein. The concentration range of fluorescein is **A**: 0.1-10000 nmol/L; **B**: 0.1-5 nmol/L.

The spectral properties of Calcein UltraGreen™ AM (fluorescent dye) perfectly match the Ex and Em wavelengths of this new fluorometer, therefore, in order to evaluate the stability of the data detected with the independently developed fluorometer, within the dynamic range of the fluorometer, a series of concentrations of the fluorescein aqueous solutions and the dye Calcein UltraGreen™ AM Tris-HCl solutions were prepared. The samples of fluorescein aqueous solution can directly detect the VRFIs of each sample. However, the VRFIs of the sample of the dye Tris-HCl solutions need to be placed at 37 °C for 20 minutes before the detection. Three parallel samples were set for each sample. The VRFIs of each sample was measured three times, and the stability of the data was evaluated by the relative standard deviation (RSD). As shown in **Table S2**, the RSD value of dye data is larger than that of fluorescein, which may be due to the characteristics of the dye itself. In general, the RSD of the detection data were less than 2 %, which is within the allowable error range. Furthermore, there is a good linear relationship between the concentration and the VRFIs of the fluorescein aqueous solutions ($y = 12.71x + 90.59$, $R^2 = 0.9996$). These results showed that the data output from the fluorometer has good reproducibility, and it could response to fluorescence intensity of the solution of fluorescein and Calcein UltraGreen™ AM stably. Therefore, the independently developed fluorometer can be used as an effective detection device.

Table S2 The reproducibility of fluorescence intensity output by the independently developed fluorometer

No.	fluorescein		dye ^a	
	Concentration (nmol/L)	RSD ^b of VRFIs ^c (%)	Concentration (μmol/L)	RSD ^b of VRFIs ^c (%)
1	10	0.81	1	1.73
2	20	0.73	3	1.15
3	50	0.73	5	1.77
4	100	0.68	8	1.01
5	250	0.66	10	0.91
6	500	0.66	15	1.09
7	800	0.56	20	0.77
8	1000	0.63	25	0.85

Note: ^a dye: Calcein UltraGreen™ AM, the VRFIs of the samples need to be placed at 37 °C for 20 minutes before the detection; ^b RSD = relative standard deviation; ^c VRFIs = the values of relative fluorescence intensity; Three parallel samples were set for each sample, and the VRFIs of each sample was measured three times.