Product Information

WonderOrange™ Protein Quantitation Kit

Catalog Number: W6006

Product Size: 100 T, 1000 T

Contents:

Component	100 T	1000 T
A. WonderOrange™ buffer, 10× (contains 2 mM sodium azide)	2.5 mL	25 mL
B. WonderOrange™ dye, 200×in DMSO	125 µL	1.25 mL
C. Bovine serum albumin (BSA) standard, 2mg/mL (contains 2 mM sodium azide)	40 µL	0.4 mL

T(Times) is based on 96 well plate calculation.

Storage

Component A: Store at room temperature; Component B: Store at room temperature and protect from light. Component C: Store at 4℃. When stored as directed, product is stable for at least 6 months.

Parameters

Ex/ Em: 480/598 nm (combined with BSA)

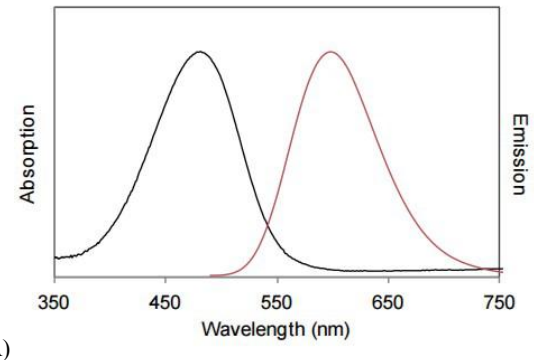

Fig.1 Spectrum of WonderOrange™ in 1×WonderOrange™ assay buffer after combined with BSA.

Description

WonderOrange™ Protein Quantitation Kit is a highly sensitive kit based on fluorescence technology for quantitative purification of protein, and its detection concentration range is 0.1-10 µg/mL. Compared with traditional methods such as BCA, Bradford or Lowry protein quantitative analysis, sensitivity of WonderOrange™ Protein Quantitation Kit is much better. In addition, compared with the NanoOrange protein quantitative analysis technology, it has better linearity and reproducibility (Fig.2). WonderOrange™ Protein Quantitation Kit can show the smallest variability between different proteins, and the fluorescence signal is stable for up to 16 hours; it applies to purified proteins and antibodies.

It is important to note that WonderOrange™ has varying degrees of tolerance to salts, buffers, detergents, or other chemicals (Table 2).

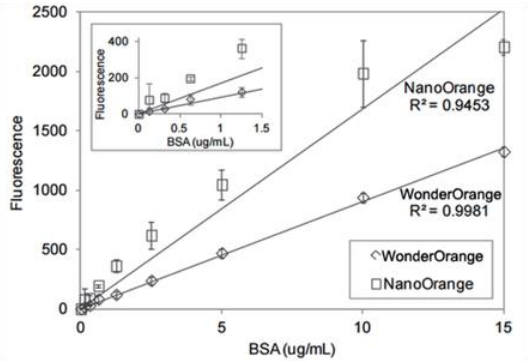

Fig.2 WonderOrange™ has better linearity and reproducibility than NanoOrange

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Protocol

1. Prepare 1 × WonderOrange™ buffer: diluted 10 × buffer for 10 times with dH_2O. For example, add 1 mL 10 × buffer to 9 mL of dH_2O.

Note: Component A is prone to precipitation. The precipitation can be dissolved by heating at 50 ℃ and will not affect the experimental results.

2. Prepare WonderOrange™ working solution: Dilute 200 × WonderOrange™ dye with 1 × WonderOrange™ buffer for 200 times. For example, add 25 µL 500 × WonderOrange™ dye to 5 mL 1 × WonderOrange™ buffer.

Note: You will need approximately 3 mL of working solution to make a standard curve (see Table 1) and 250 µL working solution for each well sample.

3. Prepare unknown sample: Add 250 µL WonderOrange™ working solution to per 10 µL sample.

Note: You may need to dilute the unknown sample to get different concentration. Sample dilution may reduce the effect of interfering substances.

4. Prepare BSA concentration required for protein standard curve, as shown in Table 1.

5. The sample and the standard protein were heated to 90 ℃ -95 ℃ for 10 min, and the process needed to be operated in dark.

6. Take out the sample and place it in the dark for cooling at room temperature. All samples were collected by short-time centrifugation.

7. Transfer 200 µL of each standard sample or unknown sample to 96 well enzyme plate, and read it with fluorescence enzyme meter. The excitation / emission wavelength is 480/598 nm.

Note: In addition, sample can be transferred to a fluorescent tube for measurement with a fluorometer. If more than 200 µL volume measurement is needed, the preparation scheme of equal scale amplification should be adopted.

Table.1 Preparation of standard curve for BSA samples

	Volume of BSA	Volume of working solution	Final concentrat-ion of BSA（µg/mL）
A	5 µL BSA std (2 mg/mL)	995 µL	10 µg/mL
B	250 µL solution A	250 µL	5 µg/mL
C	250 µL solution B	250 µL	2.5 µg/mL
D	200 µL solution C	300 µL	1 µg/mL
E	250 µL solution D	250 µL	0.5 µg/mL
F	250 µL solution E	250 µL	0.25 µg/mL
G	100 µL solution F	150 µL	0.1 µg/mL
H	0 mL	250 µL	0 µg/mL

Table.2 Tolerance to chemicals of WonderOrange™

Chemical compound	Maximum tolerance concentration
SDS	0.01%
Triton X-100	Below 0.001%
Tween 20	Below 0.001%
CHAPS	Below 0.001%
Sodium deoxycholate (DOC)	Below 0.001%
Urea	10 mM
DTT	100 mM
beta-ME	0.1%
Ammonium sulfate	1 mM
Sodium azide	2 mM
Imidazole	50 mM
DNA	10ug/mL
EDTA	1mM
Sucrose	10 mM (0.34%)
Glycerol	1%
PBS	0.02X
NaCl	1 mM
$CaCl_2$	0.01 mM
$MgCl_2$	0.2 mM

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