Product code: CTSD001-100

SangDye 3.1 Mix contents and storage:

Contents	Volume	Storage
SangDye 3.1 Mix	50 µL	-20°C to -80°C
5x Sequencing Buffer	1000 µL	4°C

Description:

SangDye 3.1 Mix is a direct replacement for Applied Biosystems' BigDye v3.1. Current BigDye v3.1 users can switch to SangDye 3.1 Mix seamlessly without changing current protocols. No new software or mobility shift file needs to be installed. No new calibration or validation is needed either.

SangDye 3.1 Mix also brings many advantages over BigDye v3.1

- **Faster reaction cycle:**
  3 min extension time, instead of 4 min, is needed for SangDye 3.1 Mix, which can be further reduced to 2 min if needed.
- **Higher dilution factor:**
  As little as 0.15 µL of SangDye 3.1 Mix can be used with success in a 5 µL total volume reaction (Fig.1)
- **Higher success rate:**
  Especially for difficult templates (Fig. 2)

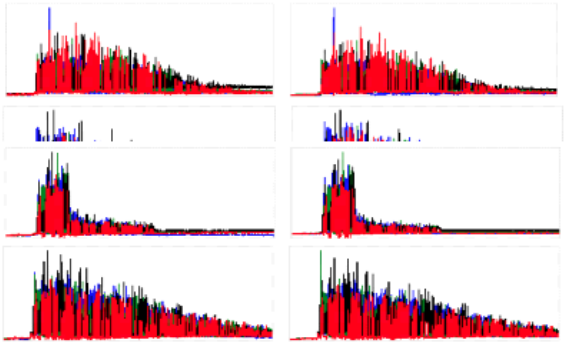

Fig. 1 Top panel: Raw sequencing signal of a normal template using 0.15 µL BigDye v3.1 in duplicate. Bottom panel: Raw sequencing signal of the same normal template using 0.15 µL SangDye 3.1 Mix. Total reaction volume was 5 µL.

Fig. 2 Top panel: Raw sequencing signal of a GT richtemplate using 0.15 µL BigDye v3.1 in duplicate.
Bottom panel: Raw sequencing signal of the same GT rich template using 0.15 µL SangDye 3.1 Mix. Total reaction volume was 5 µL.

Protocol:

This protocol only covers the reaction set up and the cycle sequencing parameters. For detailed instructions on how to prepare the templates, template concentrations, purity, and sequencing reaction purification etc. Please keep your current protocol unchanged or refer to Applied Biosystems. manual.

1. Set up reactions in 200 µL single PCR tube or 96 and 384 PCR plates according to the following formula. We only recommend 5 µL and 10 µL reaction volume. Please read the footnotes carefully.

Component	Standard reaction (5 µL)	Standard reaction (10 µL)
Template	2 µL[1],[2]	2 µL[1],[2]
Primer (5 pM)	1 µL	1 µL
5x Sequencing Buffer [3]	0.85 µL	1.7 µL
SangDye 3.1 Mix [4] [5]	0.15 µL	0.3 µL
Water [5]	1 µL	5 µL
Total volume	5 µL	10 µL

[1] **e.g.,** 150-300 ng/µL of dsDNA

[2] Concentration of template may affect volume, if template volume differs, adjust the volume of water in the reaction mix.

[3] The volume of 5x buffer needed per reaction is determined by the following formula:

$$\frac{5 \times (\text{Volume of 5x buffer} + \text{Volume of Terminator mix})}{\text{Total reaction volume}}$$

[4] Maximum of 0.5 µL or 1.0 µL SangDye 3.1 Mix are recommended for 5 µL or 10 µL reactions respectively. Using more than 0.5 µL or 1.0 µL SangDye 3.1 Mix gives less than favorable results.

[5] Alternatively, ready mix cocktail can be made by mixing 100 volumes of water, 85 volumes of 5x Sequencing Buffer and 15 volumes of SangDye 3.1 Mix. Use 2 µL or 4 µL of the ready mix for each reaction of 5 µL or 10 µL, respectively.

2 . Perform Cycle Sequencing:

Parameter	Stage/ Step				
	Incubate	32 cycles			hold
		denature	anneal	extend	
Temperature (°C)	96	96	50	60	4
Time (min)	01:00	00:10	00:10	03:00	∞

[1] Research showed longer anneal time facilitates sequencing GT rich templates.

[2] Extension for 3 minutes allows reading length of 1000bp - 1200bp. Shorter extension times can be used for short templates.