Accuris™ SmartDye™ Terminator v3.1 Cycle Sequencing Mix

Description

Accuris SmartDye Terminator v3.1 Cycle Sequencing mix is a direct replacement for Applied BioSystem™ BigDye™ v3.1. No changes in protocol are needed and no new software or mobility shift file needs to be installed in the system. There is no need for new calibration or validation.

SmartDye Advantages

- **Faster reaction cycle.** A 3 minute extension time is utilized instead of the 4 mintues required by BigDye. This can be reduced to 2 minutes for many templates.
- **Higher dilution factor.** As little as 0.15µl of mix can be used in a 5µl reaction volume.
- **Higher success rate with difficult templates.**

Storage

Upon receipt, immediately store the SmartDye Mix at-20°C to -80°C. The 5X sequencing buffer should be stored at 4°C to -20°C. Avoid excessive freeze/thaw cycles. Protect dye from light. If needed, reagents may be aliquotted into smaller amounts. Before use, thaw the frozen stocks on ice, mix thoroughly and centrifuge briefly to bring all the mix to the bottom of the tube.

Limitations of Use

For research purposes only. Not intended for therapeutic or diagnostic use.

Accuris is not responsible for consequential or incidental damages, whether direct or indirect, resulting from use of this product. Accuris guarantees the performance of this product as described when used in accordance with these instructions. It is the responsibility of the purchaser to determine the suitability of this product for their particular application.

General Guidelines

This protocol covers the reaction set up and 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 your instrument manual.

1. Reactions can be set up in 0.2ml single PCR tubes or 96 and 384 well plates according to the formula below. We recommend 5µl or 10µl total reaction volumes. Please read the footnotes carfully.

Component	5µl Reaction	10µl Reaction
Template [1],[2]	2µl	2µl
Primer (5µM)	1µl	1µl
5X Seq Buffer [3],[5]	0.85µl	1.7µl
SmartDye Mix [4],[5]	0.15µl	0.3µl
Water	1µl	5µl
Total Volume	5µl	10µl

[1] 150-300ng/µl of dsDNA
[2] Concentration of template may affect volume. If template volume differs, adjust the amount of water added to keep final volume the same.
[3] The volume of 5X buffer needed per reaction is determined by the following formula:

$$\frac{5X\ (\text{Volume of 5X buffer} + \text{Volume of Terminator mix})}{\text{Total reaction volume}} = 1$$

[4] A maximum of 0.5µl or 1.0µl of SmartDye Mix is recommended for 5µl and 10µl reactions respectively. Using more may give less than favorable results.
[5] A ready mix cocktail can be made by mixing 100 volumes of water, 85 volumes of 5X sequencing buffer and 15 volumes of SmartDye Mix. Use 2µl or 4µl of the ready mix for each 5µl or 10µl reation.

2. Perform the cycle sequencing in a thermal cycler:

Step	*Temperature*	*Time*
Initial denaturation	94°C	1 minute
30 cycles	94°C	10 seconds
	50°C	10 seconds [1]
	60°C	3 minutes [2]
Hold	4°C	Hold

[1] Research has shown that longer annealing time facilitates sequencing through GC rich templates.
[2] Extension for 3 minutes allows reading length of 1000bp - 1200bp. Shorter extension times can be used for shorter templates.

Package contents and reordering

Accuris SmartDye Terminator v3.1 Cycle Sequencing Kit is supplied with buffer, control and primer.

24 Reactions
Catalog number PR6000-24
Includes 200µl of dye, 1ml 5X buffer

100 Reactions
Catalog number PR6000-100
Includes 800µl of dye, 1ml 5X buffer

1,000 Reactions
Catalog number PR6000-1K
Includes 4x2ml of dye, 12ml 5X buffer

5,000 Reactions
Catalog number PR6000-5K
Includes 2x20ml of dye, 56ml 5X buffer

25,000 Reactions
Catalog number PR6000-25K
Includes 10x20ml of dye, 466ml 5X buffer

