

BroadDye Terminator V3.5 Cycle Sequencing Kit

The BroadDye Terminator V3.5 Cycle Sequencing Kit provides pre-mixed reagents for Sanger sequencing reactions.

Description:

This kit uses four different fluorescent dyes to label ddNTPs, which are added sequentially to the primer through a cycle sequencing reaction. The kit reagents are suitable for performing fluorescence-based cycle sequencing reactions on single-stranded or double-stranded DNA templates, on PCR fragments, and on large templates (for example, BAC clones).

Features:

With enhanced robustness and better dye mobility characteristics, the BroadDye Terminator V3.5 Cycle Sequencing Kit can be used in a wide range of applications with the following features:

- Long read lengths.
- Better performance reading through GC rich regions.
- Longer reads with more uniform peak heights.
- Improved productivity for less cost.

Applications:

- De novo sequencing
- Resequencing
- Sequencing difficult templates
- Long-read sequencing
- Sequencing across all template types (plasmids, PCR products)
- Mixed-base detection
- Sequencing short PCR products using rapid electrophoresis run modules

Platforms:

- 3730/3730xl DNA Analyzer
- 3500/3500xL Genetic Analyzer
- 3130/3130xl Genetic Analyzer
- 310 Genetic Analyzer
- Locus Sector 16
- Honor 1624/1816/1824

Template Compatibility:

Plasmid DNA ($\leq 15\text{Kb}$)

Single Stranded DNA

Lambda DNA

Genomic DNA (Bacterial)

PCR Amplicons

Dye set

The kits are optimized to run with Dye Set Z for BroadDye Terminator v3.5.

BroadDye Terminator V3.5 Cycle Sequencing Kit contents-standard:

Cat. No.	Contents	Quantity	Storage
BDT-100	BDT V3.5 Mix	800ul	
	5X Seq buffer	2.4ml	May be stored at 4°C
	pGEM_3Zf(+) Control DNA	1 tube	
	M13(-21) Primer	1 tube	
BDT-1000	BDT V3.5 Mix	8ml	
	BDT V3.5 5X Seq buffer	24ml	May be stored at 4°C
	pGEM_3Zf(+) Control DNA	1 tube	
	M13(-21) Primer	1 tube	
BDT-5000	BDT V3.5 Mix	40ml	
	BDT V3.5 5X Seq buffer	120ml	May be stored at 4°C
	pGEM_3Zf(+) Control DNA	1 tube	
	M13(-21) Primer	1 tube	

Storage: Store kit at -15°C to -25°C. And avoid repeated freeze-thaw cycles.

Regulatory Statement: For Research Use Only. Not for use in diagnostic procedures.

Instructions:

Please add DNA Template according to the following instructions.

DNA Template	Quantity	
PCR products	100-200bp	1-3ng
	200-500bp	3-10ng
	500-1000bp	5-20ng
	1000-2000bp	10-40ng
	>2000bp	20-50ng
Single-stranded DNA	25-50ng	
Double-stranded DNA	150-300ng	
Cosmid, BAC	0.5-1.0µg	
Bacterial genomic DNA	2-3µg	

Construct a sequencing PCR system according to the following volume:

Reagent	10 μ L reaction system (1/8X reaction concentration)	10 μ L reaction system (1/16X reaction concentration)
H ₂ O	5.7 μ L	5.8 μ L
-21 M13 Control Primers (3.2 μ M)	1 μ L	1 μ L
BDT UltraSeq Master Mix	0.5 μ L	0.3 μ L
5x Sequencing Reaction Buffer	1.8 μ L	1.9 μ L
pGEM-3Zf(+)-Control (200ng/ μ L)	1 μ L	1 μ L
Total volume	10 μ L	10 μ L

The operational cycle of sequencing reaction:

Parameter	Stage/step				
	Incubate	30 cycles			Hold
		Denature	Anneal	Extend	
Ramp rate	—	1°C/second			
Temperature	96°C	96°C	50°C	60°C	4°C
Time (mm:ss)	1:00	00:10	00:05	4:00	Hold until ready to purify.