QuinDye™ Terminator

Catalog No: CPR00001,CPR00002,CPR00003,CPR00004

For Research Use Only. Not for use in diagnostic procedures.

Product Description

QuinDye™ Terminator Kit provides premixed reagents for Sanger sequencing reactions. The kit includes Dye Terminator and a 5x buffer, formulated to deliver long, high-quality reads with uniform peak heights and to efficiently read through complex sequencing structures.

Components/Kit Information

Catalog No	CPR00001	CPR00002	CPR00003	CPR00004
Reactions	24 Reactions	100 Reactions	1000 Reactions	5000 Reactions
QuinDye Sequencing Mix	192 µL*1	800 µL*1	800 µL*10	20 mL*2
pGEM control DNA	10 µL*1	10 µL*1	250 µL*1	250 µL*2
M13 (-21) control primer	10 µL*1	10 µL*1	200 µL*1	200 µL*2
QuinDye 5x Sequencing Buffer	1 mL*1	1 mL*2	12 mL*1	28 mL*2

Storage Condition

Store QuinDye Sequencing Mix, pGEM control DNA, and M13 (-21) control primer at –30°C to –10°C in a frost-free freezer. Protect the sequence mix from light. Store QuinDye 5x Sequencing Buffer at 4°C in a frost-free freezer

REAGENTS to be supplied by the User

- Reagent Alcohol, EtOH
- Nuclease free H2O

Compatible Sequencing Instruments

- 3730 DNA Analyzer
- 3500 Genetic Analyzer
- 3130 Genetic Analyzer

Procedure

1. Prepare DNA Template

DNA template	Recommended Quantities
PCR product	10-20ng per kb
Single-stranded DNA	25–50 ng
Double-stranded DNA	150–300 ng
Cosmid, BAC	0.5–1.0 µg
Bacterial genomic DNA	2–3 µg

Notice, the sequencing templates should be purified before use in sequencing reactions.

2. Perform cycle sequencing

2.1 Set Up the Sequencing Reactions

2.1.1 Completely thaw the components of the QuinDye™ Terminator Kit and your primers, then store them on ice.

2.1.2 Vortex the tubes for 2 to 3 seconds, then centrifuge 2 to 3 seconds to collect contents at the bottom of the tubes.

2.1.3 Add components as indicated. This setup is for standard 20 µL reactions, but the volume can be reduced to 10 µL in 384-well plate. In this case, maintain the same primer concentration and volume as used in the 20 µL reactions.

2.1.4 Seal the plate and vortex for 2 to 3 seconds, then briefly centrifuge to collect contents at the bottom of the wells (5 to 10 seconds) at 1,000 × *g*.

Component	Quantity per reaction	Example Forward	Example Reverse
QuinDye Sequencing Mix	8 µL	8 µL	8 µL
Forward primer (3 µM)	1 µL	1 µL	-
Reverse primer (3 µM)		-	1 µL
Deionized water (RNase/DNase-free)	Varies based on template and primer volume	9 µL	9 µL
Template	See above template quantity*	2 µL	2 µL
Total volume	**20 µL**	**20 µL**	**20 µL**

2.2 Run the sequencing reactions

2.2.1 Place the tubes or plate(s) in a PCR machine and set the volume.

2.2.2 Perform cycle sequencing:

Parameter	Stage/step				
	Incubate	Cycling (25 cycles)			Hold
		Denature	Anneal	Extend	
Ramp rate	—	1°C/second			
Temperature	96°C	96°C	50°C	60°C	4°C
Time (mm:ss)	01:00	00:10	00:05	04:00*	Until ready to purify

* Shorter extension times can be used for short templates.

Using QuinDye™ Terminator 5X Sequencing Buffer to dilute sequencing reactions

Some cycle sequence reactions may be optimized using diluted QuinDye™ Sequencing Mix. The QuinDye 5x Sequencing Buffer can be used to dilute the QuinDye Sequencing Mix.

Note: If you use the QuinDye 5X Sequencing Buffer without optimization, the quality of the sequence may deteriorate. We can not guarantee the performance of QuinDye™ chemistry when it is diluted.

An example of a 0.5x diluted sequencing reaction is shown below:

Component	Quantity per reaction	Example Forward	Example Reverse
QuinDye Sequencing Mix	4 µL	4 µL	4 µL
QuinDye 5x Sequencing Buffer	2 µL	2 µL	2 µL
Forward primer (3 µM)	1 µL	1 µL	-
Reverse primer (3 µM)		-	1 µL
Deionized water (RNase/DNase-free)	Varies based on template and primer volume	11 µL	11 µL
Template	See above Template quantity*	2 µL	2 µL
Total volume	**20 µL**	**20 µL**	**20 µL**

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

IMPORTANT! Protect dye terminators from light. Cover the reaction mix and sequencing plates with aluminum foil before use.

For technical support, please visit Quintara Bioscience or email technical support team sales.us@quintarabio.com

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