

ABgene® vs competitor Dye Terminator Removal kits

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Dye terminator removal kits provide a convenient package to perform the essential removal of unbound fluorescently-labelled dideoxy-ribonucleotides (ddNTPs), excess salt and primers from sequencing reactions prior to sequence analysis. Sequencing reactions are passed through a separation matrix that retains the shorter ddNTPs and primers, while larger PCR products are recovered in the filtrate. This has an advantage over ethanol precipitation methods where residual dye terminators may still be present in the sample¹. If an excess of free fluorescently-labelled dye terminators remain in the purified sequencing reaction, they will cause large peaks or 'dye blobs' that occur at specific places on migration through the sequencing gel. These dye blobs can obscure genuine base readings, especially near the beginning of the sequence, and adversely affect the overall data quality.

ABgene®'s Dye Terminator Removal Kit was evaluated against similar products from 3 other major competitors in terms of signal strength, unincorporated dye terminator removal and sequence quality.

Method

Reactions were prepared in order to sequence a section of pGEM®-3Zf(+) plasmid (Promega) using the -21 M13 forward primer, as supplied with the 'ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit' versions 2.0 and 3.0 (Applied Biosystems). Cycle sequencing was performed according to the manufacturer's instructions and identical reactions were pooled after thermal cycling.

Each dye terminator removal plate was used following the supplier's recommendations. The protocols were very similar, employing centrifugation steps between 850–1000g for 3–5 minutes. For all kits tested, an initial centrifugation was performed and the flow-through discarded. Replicate sample volumes between 10µl and 20µl were slowly applied to each well and subsequent centrifugation allowed the sample to be collected in a V-bottomed plate. When using competitor kit 'A' (see results section) with the BigDye™ 3.0 kit, it was necessary to include an extra wash stage with 150µl of 1mM EDTA (pH 8) prior to adding the sample. The purified sequencing reactions were all

vacuum-dried before analysis on the ABI PRISM® 377 automatic sequencer (Applied Biosystems). Due to computer-generated errors, the returned sequences were manually edited where necessary, then compared to the original vector sequence using the BLAST program (Basic Local Alignment Search Tool²) at www.ncbi.nlm.nih.gov.

Results & Discussion

The results in Table 1 show mean values from pooled triplicate 15µl samples using the BigDye™ version 3.0 cycle sequencing kit (Applied Biosystems). The 'start' of the read was the point 3' from the primer at which the sequencing instrument could clearly distinguish between bases. Subsequent incorrectly matched nucleotides were termed 'misreads' and their occurrence in the ensuing 400, 500 and 600 nucleotides was recorded. The presence of 'dye blobs' that interfered with base calling were noted.

The mean signal intensity values for each labelled base are shown as obtained from the automated sequence analysis.

Company	Sample volume added to plate (µl)	Dye terminator removal		Sequence quality			Signal intensity (a.u)				
		Dye Blob y = yes n = no	Sequence nucleotide start number	Misreads in 1st 400 matched nucleotides	Misreads in 1st 500 matched nucleotides	Misreads in 1st 600 matched nucleotides	G	A	T	C	Total
ABgene®	15	n	35	5	6	9	309	174	158	151	792
Competitor A	15	y	37	7	7	11	192	116	91	101	500
Competitor Q	15	y	36	5	5	9	179	84	88	76	427
Competitor E	15	n	43	4	5	9	199	105	92	93	489

Table 1: Analysis of sequence quality, dye terminator removal and signal strength using several commercial dye terminator removal kits.

ABgene®'s kit gave superior results over the competitor kits as no dye blobs occurred. In addition, the ABgene® kit gave the highest total base signal strength (acceptable values > 400³). A similar distribution of results was observed when using 10µl and 20µl sample volumes, however, using lower volumes in competitor kits frequently resulted in signal intensities less than 100 per base. Although these may be of good quality, errors can be introduced due to a low signal-to-noise ratio³. No dye blobs were observed when using the ABgene® kit with any of the sample volumes tested.

In general, increased signal intensities were observed using BigDye™ version 2.0 over version 3.0 (results not shown) and no manual editing was necessary for version 2.0. This may be due to the difference in sequencing chemistries.

Product Description

ABgene®'s Dye Terminator Removal Kit provides a complete package, including 96-well separation plates, wash plates and sample collection plates. The separation

plates contain a pre-hydrated gel filtration matrix and a novel polyethylene frit for enhanced performance. To ensure 100% protection against leakage the filtration plates are heat sealed top and bottom. The quick



filtration protocol of 15 minutes is up to 6 times faster than ethanol precipitation, thus enabling faster sample processing.

For further information please contact ABgene®.

References

1. ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit for -21 M13 and M13 Rev Primers: Protocol Rev A, 4390036A.
2. Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman D. J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*. **25**:3389-3402.
3. http://cancer.duke.edu/DNA/Sequencing/data_int.asp

Cat. No.	Description	Quantity
AB-0943/a	Dye Terminator Removal Kit (MegaBACE™ compatible)	4 x 96 preps
AB-0943/b	Dye Terminator Removal Kit (MegaBACE™ compatible)	24 x 96 preps
SP-0411/a	Dye Terminator Removal Kit (ABI PRISM® compatible*)	4 x 96 preps
SP-0411/b	Dye Terminator Removal Kit (ABI PRISM® compatible*)	24 x 96 preps

* Sample must be transferred manually into ABI compatible plate.