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Dye Terminator Clean-Up Kits
Product Information

Product: Dye Terminator Clean-Up Kits
Protocol No.: 60.002
Storage & Stability: Stable for 1 year at Room Temperature

PRODUCT NO.	DESCRIPTION	UNIT
DTC-96-01	96-Well Dye Terminator Clean-Up Kit	Starter Kit (1 x 96)
DTC-96-10		Mini Kit (10 x 96)
DTC-96-50		Midi Kit (50 x 96)
DTC-96-100		Maxi Kit (100 x 96)
DTC-384-01	384-Well Dye Terminator Clean-Up Kit	Starter Kit (1 x 384)
DTC-384-10		Mini Kit (10 x 384)
DTC-384-50		Midi Kit (50 x 384)
DTC-384-100		Maxi Kit (100 x 384)

Introduction
Congratulations on taking a big step towards improving the economies of scale, quality and speed of your genomics research. This is a complete set of protocols outlining the steps and principles needed to use the Dye Terminator Clean-Up Kit.

Product Description
The Dye Terminator Clean-Up Kits are an advanced purification system containing sophisticated separation buffers and either 96- or 384-well purification plates. Use of our Dye Terminator Clean-Up Kits will increase the quality of your DNA sequence data by removing unwanted salts, enzymes, primers, unincorporated dyes and nucleotides, and other contaminants that diminish the quality of DNA sequence reads. Users will appreciate the following features:

- Supports all gel and capillary electrophoresis sequencing platforms
- Industrial sequencing capacity (20 megabases per Maxi Kit)
- Reduces cost
- Longer reads, 10-20% more sequence data per run
- High-throughput (10,000 samples per day manually)
- Compatible with all 96- and 384-well thermal cycles
- Superior chemistry provides 99+% purity
- Dye removal produces clean sequence data
- Minimizes wear and clogging of costly glass capillaries
- High yield produces stronger sequencing signals
- No alcohol precipitations required
- Arrives ready to use, no buffer or column preparation required

Kit Contents:

96 Well Kits	*384 Well Kits*
Starter Kit (1 x 96)	**Starter Kit (1 x 384)**
Clean-Up Filter 96-well (1)	Clean-Up Filter 384-well (1)
Wash Microplate 96-well w/o cover (1)	Wash Microplate 384-well w/o cover (1)
Sample Microplate 96-well with cover (1)	Sample Microplate 384-well with cover (1)
Binding Buffer (5 ml)	Binding Buffer (25 ml)
Wash Buffer (30 ml)	Wash Buffer (100 ml)
Mini Kit (10 x 96)	**Mini Kit (10 x 384)**
Clean-Up Filter 96-well (10)	Clean-Up Filter 384-well (10)
Wash Microplate 96-well w/o cover (10)	Wash Microplate 384-well w/o cover (10)
Sample Microplate 96-well with cover (10)	Sample Microplate 384-well with cover (10)
Binding Buffer (60 ml)	Binding Buffer (200 ml)
Wash Buffer (250 ml)	Wash Buffer (1 x 1 liter)
Midi Kit (50 x 96)	**Midi Kit (50 x 384)**
Clean-Up Filter 96-well (50)	Clean-Up Filter 384-well (50)
Wash Microplate 96-well w/o cover (50)	Wash Microplate 384-well w/o cover (50)
Sample Microplate 96-well with cover (50)	Sample Microplate 384-well with cover (50)
Binding Buffer (300 ml)	Binding Buffer (1 x 1 liter)
Wash Buffer (1.5 liter)	Wash Buffer (5 x 1 liter)
Maxi Kit (100 x 96)	**Maxi Kit (100 x 384)**
Clean-Up Filter 96-well (100)	Clean-Up Filter 384-well (100)
Wash Microplate 96-well w/o cover (100)	Wash Microplate 384-well w/o cover (100)
Sample Microplate 96-well with cover (100)	Sample Microplate 384-well with cover (100)
Binding Buffer (500 ml)	Binding Buffer (2 x 1 liter)
Wash Buffer (3 x 1 liter)	Wash Buffer (10 x 1 liter)

Reagent and Equipment Requirements

- Dye terminator kit
- PCR 96- or 384-well plates
- Thermal cycler
- Vacuum certrifuge
- Vacuum manifold

Short Protocol

1. Set up 5-25 µl dye Terminator sequencing reactions.
2. Amplify sequence templates by thermal cycling.
3. Add 2 volumes (10-50 µl) of Binding Buffer per well.
4. Mix extension products and Binding Buffer by pipetting up and down 10 times.
5. Pipette each sample into an individual well of a 96- or 384-well Clean-Up Filter.
6. Filter samples through Clean-Up Filter by applying a gentle vacuum.
7. Wash Clean-Up Filter 3 times with 80 µl per well of Wash Buffer.

8. Apply strong vacuum for 5 minutes to dry Clean-Up Filter membrane.

9. Remove Wash Buffer by centrifugation into the Wash Microplate (5 minutes at 500 x g).

10. Add 50 µl H_20 to each well of the Clean-Up Filter.

11. Incubate Clean-Up Filter for 1 minutes at room temperature.

12. Elute purified products into a 96- or 384-well Sample Microplate (5 minutes at 500 x g).

13. Evaporate samples to dryness by vacuum centrifugation (20 minutes).

14. Re-suspend each sample in 3.0 µl loading buffer and load onto sequencing gel.

Short Protocol (centrifugation only)

1. Set up 5-25 µl dye Terminator sequencing reactions.

2. Amplify sequencing templates by thermal cycling.

3. Add 2 volumes (10-50 µl) of Binding Buffer per well.

4. Mix extension products and Binding Buffer by pipetting up and down 10 times.

5. Pipette each sample into an individual well of a 96- or 384-well Clean-Up Filter.

6. Centrifuge for 1 minute (500 x g) to remove unbound material.

7. Add 80 µl per well of Wash Buffer and centrifuge for 1 minute (500 x g).

8. Wash Clean-Up Filter two more times with 80 µl Wash Buffer (as in Step 7).

9. Centrifuge for 5 minutes (500 x g) to remove residual Wash Buffer.

10. Add 50 µl H_20 to each well of the Clean-Up Filter.

11. Incubate Clean-Up Filter for 1 minute at room temperature.

12. Elute purified products into a 96- or 384-well Sample Microplate by centrifugation for 5 minutes (500xg).

13. Evaporate samples to dryness by vacuum centrifugation (20-40 minutes).

14. Re-suspend each sample in 3.0 µl loading buffer and load onto sequencing gel.

Complete Protocol

1. Set up dye Terminator sequencing reactions in the appropriate volume (5-25 µl). For example the standard sequencing reaction using the ABI Prism Big Dye Terminator Kit is 20 µl, which is made by mixing 8.0 µl of Terminator Ready Reaction Mix and 12.0 µl of template and primer. To conserve reagents, smaller volume reactions (5-10 µl) can be used. Typical amounts of template are 50-200 ng for single-stranded DNA, 200-500 ng for double-stranded DNA, and 50-100 ng of a 1 kb PCR product. Typical amounts of primer are 2-5 pmoles. Custom primers may require some optimization.

2. Amplify sequence templates by thermal cycling in a 96- or 384-well thermal cycler. Set the thermal cycler to the appropriate volume (5-25 µl) and cycle for 25 rounds. For the ABI Prism Big Dye Terminators in a GeneAmp 9600, the recommended regime is 96°C (10 sec), 50°C (5 sec), 60°C (4 minutes). Different sequencing chemistries and thermal cyclers may require adjustments in the thermal cycling conditions. After 25 rounds of amplification, hold the temperature at 4°C until ready to purify.

3. After thermal cycling, remove the 96-well or 384-well plates from the thermal cycler and add 2 volumes (10-50 µl) of Dye Terminator Clean-Up Binding Buffer to each well. The Binding Buffer has a pale green appearance. Be careful to use Binding Buffer and NOT Wash Buffer, which is clear in appearance.

4. Mix the sequencing extension products and Dye Terminator Clean-Up Binding Buffer by pipetting the mixture up and down 10 times. Care must be taken to avoid splashing samples between wells. This will diminish the quality of the sequence data.

5. After the samples are mixed, pipette each sample into an individual well of a 96- or 384-well Clean-Up Filter positioned on a vacuum filtration apparatus. Make sure that each sample is transferred correctly from the thermal cycling plate into the Clean-Up Filter. Incorrect transfer will lead to errors in sample tracking! For best results, add the sample directly onto the Clean-Up Filter membrane, instead of onto the sides of the Clean-Up Filter. Samples added to the sides of the Clean-Up Filter wells will reduce the efficiency of the binding and wash steps.

6. Once the samples have been transferred from the thermal cycling plates into the Clean-Up Filter, filter the samples through Clean-Up Filter by applying a gentle vacuum. The sequencing products will remain bound to the filter, and unwanted components such as primers, enzymes, salts and unincorporated dyes and nucleotides will pass through the filter into to waste container. Once the 10-50 µl sample volume has passed through the filter, turn the vacuum off. This will avoid excessive drying of unwanted components onto the Clean-Up Filter membrane prior to the wash steps.

7. Wash the Clean-Up Filter three times with 80 µl per well of Dye Terminator Clean-Up Wash Buffer. The Wash Buffer contains cleansing components that remove residual contaminants such as primers, enzymes, salts and unincorporated dyes and nucleotides from the bound sequencing products. Each of the three wash steps is performed by adding 80 µl of Wash Buffer, then applying a gentle vacuum until the 80 µl volume of Wash Buffer passes through the filter into the waste container. The vacuum should be left off between rounds of washing to avoid excessive drying of sequencing product onto the filter during the wash steps.

8. After the third wash step, apply a strong vacuum for 5 minutes to dry the Clean-Up Filter membrane. This removes excess Wash Buffer from the Clean-Up Filter membrane, which can reduce yield and compromise the quality of the sequence data. Dying also increases the yield of the purified extension product during the elution step.

9. After the 5 minutes drying step, remove residual Wash Buffer by centrifugation (5 minutes at 500 x g). This is done by placing the Clean-Up Filter containing the samples on top of a 96- or 384-well Wash Microplate and centrifuging the two plates such that the residual Wash Buffer empties into the Wash Microplate (bottom plate). Following centrifugation, discard the Wash Microplate (lower plate) containing the residual Wash Buffer.

10. Place the Clean-Up Filter containing the purified samples on top of a 96- or 348-well Sample Microplate and add 50 µl H_20 to each well. Make sure to pipette the H_20 directly onto the surface of the Clean-Up Filter membrane to allow wetting. Adding the H_20 to the side of Clean-Up Filter wells reduces wetting of the filter and may lead to sample loss.

11. Incubate the Clean-Up Filter on top of the Sample Microplate for 1 minutes at room temperature. This allows the purified sequencing product to re-hydrate. Eliminating the 1 minute incubation may lead to sample loss. Longer incubation times (1-10 minutes) will not effect sample yield.

12. After the 1 minute incubation, transfer the Clean-Up Filter and the Sample Microplate (lower plate) into a centrifuge and spin for 5 minutes at 500 x g to elute the purified sequencing products into the 96- or 384-well Sample Microplate. Care must be taken to align the Clean-Up Filter (top) and Sample Microplate (bottom) with respect to each other, so that the samples from the Clean-Up Filter transfer accurately into the sample Microplate. After the 5 minutes centrifugation step, remove and discard the Clean-Up Filter (upper). The purified sequencing samples are contained in the Sample Microplate (lower) in approximately 50 µl of H_20.

13. Transfer the 96- or 384-well Sample Microplates containing the sequencing products into a vacuum centrifuge and evaporate samples to dryness by vacuum centrifugation. This usually takes ~20 minutes with heating at 65°C. Longer periods of time (60 minutes) may be required if ambient temperatures are used. The dried sequencing samples will be present on the bottom of each well of the 96-well or 384-well Sample Microplate.

14. Add 3.0 µl loading buffer to each well of the 96-well or 384-well Sample Microplate, containing the purified sequencing sample. Re-suspend each dye Terminator sequencing sample in 3.0 µl loading buffer by pipetting up and down 10 times. Load the entire 3.0 µl sample onto a gel and carry out electrophoresis according to the instructions of the manufacturer.

Troubleshooting Tips

Poor sequence data early in read due to contaminating dyes in purified extension products:

- Extension products drying on the Clean-Up Filter membrane prior to the wash steps
- Incomplete washing of extension products bound to Clean-Up Filter membrane

Weak fluorescent signal in sequence traces due to low yield of extension product::

- Poor amplification due to inferior templates or primers
- Poor binding of extension products to Clean-Up Filter membrane

For in vitro research use only.

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Literature Cited

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