A&A BIOTECHNOLOGY
innovating life science

ExTerminator

Nucleotide dye terminators removal kit for DNA cycle sequencing reaction samples.

version 1117

50 isolations, 250 isolations

Cat. # 444-50, 444-250

Maximum binding capacity of the minicolumn is 10 µg.

For R&D use only

Kit Contents

Component	50 isolations	250 isolations	Store at
Minicolumns	50 pcs	250 pcs	Room Temp.
1.5 ml tubes	50 pcs	250 pcs	Room Temp.
WP bind/wash solution	30 ml	140 ml	Room Temp.
Mix Blue	300 µl	1500 µl	Room Temp.
Sterile water (nuclease free, DEPC treated)	8 ml	15 ml	from -20 °C to +20 °C

Equipment and materials necessary for nucleotide terminator removal that are not included in kit

1. Cycle sequencing reaction mixture
2. Benchtop microcentrifuge

NOTE:
Before you start working, we recommend cleaning the work surface using LabZAP™ product (cat. # 040-500)

A&A Biotechnology provides one year guarantee on this kit

The company does not guarantee correct performance of this kit in the event of:
not adhering to the supplied protocol
not recommended use of equipment and materials
the use of other reagents than recommended or which are not a component of the kit
the use of expired or improperly stored reagents and columns

Terminators removal protocol

1. Add 5 µl of Mix Blue to cycle sequencing mixture (performed in 10-20 µl).

   Note: If cycle sequencing reaction is less than 10 µl add an appropriate volume of sterile water to reach the final volume of 10 µl.

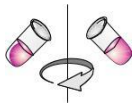
2. Add 100 µl of WP bind/wash solution Mix

   by pipeting.

3. Apply samples onto the minicolumns.

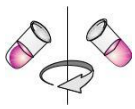
4. Centrifuge for 30 s at 12 000-14 000 RPM.

   Note: light blue colour of the minicolumn membrane is a result of efficient precipitation of sequencing products.



5. Apply 400 µl of WP bind/wash solution onto the minicolumns.

6. Centrifuge for 2 min at 12 000-14 000 RPM.



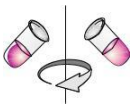
7. Transfer the minicolumns to new 1,5 ml tubes (included).

8. Add sterile water directly onto the minicolumns resin: - -
capillary sequencer - 25 µl of sterile water.
- slab gel sequencer - 50 µl of sterile water.

While applying water onto the minicolumn be sure that liquid is applied directly onto the resin. If some water stay on the minicolumn wall the elution will be less effective.

9. Incubate for 2 min at room temp.

10. Centrifuge for 1 min at 12 000-14 000 RPM.



11. Clear light blue appearance of the eluted samples confirms the correct isolation of cycle sequencing DNA products.
Blue colour of the sample does not affect the readout of the DNA sequence.

Capillary sequencer:

The samples are ready for thermal denaturation. Thermal denaturation can be directly proceed in the tube with the column.

Slab gel sequencer:

Dry up the samples using a vacuum dryer and dissolve in an appropriate amount of loading buffer.

12. Store the samples at -20 °C.

Safety Information

DANGER

WP bind/wash solution

H225 Highly flammable liquid and vapour.
H319 Cuses serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapours.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.